Dynamics of gas formation during ensilage

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Introduction

The formation of a whole spectrum of gasses occurs during ensiling process. The formation of gasses is undesirable, because it is often a sign of undesirable processes in silages, and causes concern about the impact on the global environment. Formation of CO_2 is the most abundant during ensiling and its volume can reach up to 80% of total gasses produced during the first 60 ensiling days (Peterson et al., 1958). Processes where CO_2 occurs as a by-product are less effective in transformation of substrate to main fermentation products which results in higher ensiling losses (McDonald et al., 1991). Therefore, the formation CO_2 can be considered as a measure of ensiling efficiency and ensiling losses. Besides CO_2, the formation of toxic N oxides also occurs during ensiling. Research concerning nitrogenous gas formation during ensiling is sporadic and often incomplete (Spoelstra, 1985). Since silage additive addition affects the fermentation pattern, the aim of the study was to monitor the formation of various gasses from silages treated with different silage additives.

Materials and Methods

A grass ley (70% timothy) was harvested on August 24th 2013, nearby Helsingborg, southern Sweden. The forage crop was directly harvested using a precision chopper (Claas Jaguar 690). Standard analyses to determine composition of the fresh forage (FF) such as dry matter (DM), buffering capacity, ash, water soluble carbohydrates (WSC), pH, neutral detergent fibre (NDF), metabolisable energy (ME), crude protein (CP), and hygienic quality (enterobacteria, clostridia spores) were performed. The forage was divided in three fractions. One was left untreated while the rest was treated with either of two silage additives; one with a bacterial inoculant (*E. faecium, L. plantarum, L. buchneri*) at the rate of 250000 cfu/g FF, and the second one with Safesil at the rate of 3 L/ton FF. Forages were ensiled in steel laboratory silos (vol. of 25 L). Each treatment consisted of 6 replicates. Bottom and lids of silos were equipped with stoppers with tubes allowing collection of gas and silage liquids. Two forms of gas collection were applied. First, the escaping gasses in three silos of each treatment were collected into Tedlers bags (Supelco), which were regularly changed. Gasses collected in bags were analysed for N_2, H_2, O_2, CO, CH_4 by gas chromatographaphy. Separation was done on a packed column (40/60 mesh, 4 m, OD 1/8) in a Perkin Elmer Clarus 580 gas chromatograph using TCD detector. The second set of three silos of each treatment was connected to distilled water baths (regularly changed) in which CO_2, NO, and NO_2 were absorbed. The gases absorbed in water were analysed using ion chromatography by use of a UV detector according to ISO 10304-1. Gas collections were done during14 days (bacterial treatment for 30 days). At the end of storage (120 days), silages samples were extracted and standard analyses (DM, volatile fatty acids, lactic acid, ethanol, pH, NDF,

ME, crude protein, lactic acid bacteria, clostridia spores, aerobic stability) were performed to determine silage quality.

Results and Discussion

The chemical and microbiological composition of the forage, prior to ensiling, is presented in Table 1. Chemical composition of fresh forage represented a common composition found in third cut grass crops in Sweden. Calculated fermentation coefficient of 39 characterizes the forage as slightly above the limit for a difficult crop to ensile (Weissbach, 1974). The analyses of microbiological contamination revealed high counts of enterobacteria and particularly of clostridia spores.

Table 1. Chemical and microbiological compositions of fresh forage (n=2)

Analyses	Unit	
DM	%	23.8
Ash	% of DM	8.6
CP	% of DM	13.8
WSC	% of DM	11.3
NDF	% of DM	55.9
ME	MJ/kg DM	10.9
Buffering capacity	g LA/100 g DM	6.0
Enterobacteria	log cfu/g FM	3.4
Clostridia spores	log cfu/g FM	4.7
pH of forage mass		6.2
Fermentation coefficient		39

DM-dry matter; FM-fresh matter; CP-crude protein; WSC-water-soluble carbohydrates; NDF-neutral detergent fiber; ME-metabolisable energy.

Results of chemical analyses of the silages displayed variation in chemical composition (Table 2). Silages treated with bacterial inoculant had significantly higher pH, propionic acid, acetic acid, and ethanol contents but lower concentration of lactic acid than other silage treatments

Table 2. Chemical composition of silages at the end of storage (n=3)

	DM	pH	NH_3-N	Lactic acid	Acetic acid	Propionic acid	Ethanol
	%		% TN	% DM			
Control	24.3	4.0	6.6	10.1	1.7	0.03	0.5
Inoculant	24.1	4.4	7.1	3.9	5.5	0.45	0.8
Safesil	25.1	4.0	6.2	8.7	1.8	<0.04	0.3
$LSD_{0.05}$		0.02	1.24	0.38	0.20	0.02	0.05
P-value		0.001	0.3	0.001	0.001	0.001	0.001

It is assumed that the effects were caused by *L. buchneri* in the silage innoculant which is known to form acetic acid at the expence of lactic acid (Reich & Kung, 2010). As a concequence of the high levels of acetic acid, silage treated with bacterial innoculant were found to be significanly more aerobicaly stable than the untreated control silage (Table 3). This study confirmed early findings (Knicky & Spörndly, 2009, 2011) that Safesil is efficient to secure a proper ensiling process and in improving the aerobic stability of silage.

Table 3. Microbiological composition and aerobic stability of silages at the end of storage (n=3).

	Time (hours) until temp. aerated silages increased 3°C	Max. temp (°C)	Max. temp. increase (°C)	pH after stab.	Yeasts (lg cfu/g)
Control	72	31.8	13.9	6.1	3.6
Inoculant	166	19.6	1	4.5	<1.7
Safesil	166	19.3	0.3	4.1	<1.7
$LSD_{0.05}$	16.8			1.37	0.32
P-value	0.001			0.02	0.001

Gas analyses revealed significantly higher formation of total gasses in control silage and bacterial innoculant treated silage than in the Safesil treated silage (Figure 1). This could be explained by differences in fermentation intensity and variation in bacterial composition among the silage treatments. A high gas formation in bacterially inoculated silages is assumed to be a consequence of addition of bacterial microflora which intensified the fermentation process. On the other hand, Safesil possesses a rather selective inhibitory property which restricts particularly undesirable fermentation processes. This probably caused a lower gas formation in Safesil treated silages. The proportion of CO_2 of total gass was 0.56 for Safesil, 0.65 for bacterial inoculant, and 0.68 for control silage and similar to Peterson et al. (1958).

All silages displayed a similar pattern in development of gas over time. The highest formation of gasses was observed approximatelly between 11-29 hours of the ensiling period. This peek of gas formation corresponds to the period of the most intensive fermentation, according to Pahlow et al. (2003). Another increase in gas formation was observed in bacterialy innoculated silages after ca. 300 hours of ensiling. This increase in CO_2 formation was observed only in these silages (Figure 2). This phenomenon is regarded to be the consequence of *L. buchneri* activity, which has the ability to convert lactic acid into acetic acid under unearobic conditions (Oude Elfering et al., 2001) and where CO_2 is formed as a bi-product of this conversion.

Due to lack of formation of ensiling liquid, it was impossible to follow degradation of nitrate during ensiling process. Neverheless, the formation of NO (Figure 3) and NO_2 (Figure 4) seemes to follow the degradation of nitrate and nitrite during fermentation, as described by Spoelstra (1985) and demonstrated by Knicky & Lingvall (2005) and Knicky & Spörndly (2009). The study of Peterson et al. (1958) showed a similar pattern of NO formation as in the present study. Surpisingly, the formation of all NO_x gasses revealed no statistical differences between Safesil and the other silages. A higher formation of NO was expected due to the presence of Na-nitrite in Safesil. The lack of N_2O measurement as well as monitoring of ammonia formation during fermentation process makes it difficult to explain the lack of variation in NO_x formation.

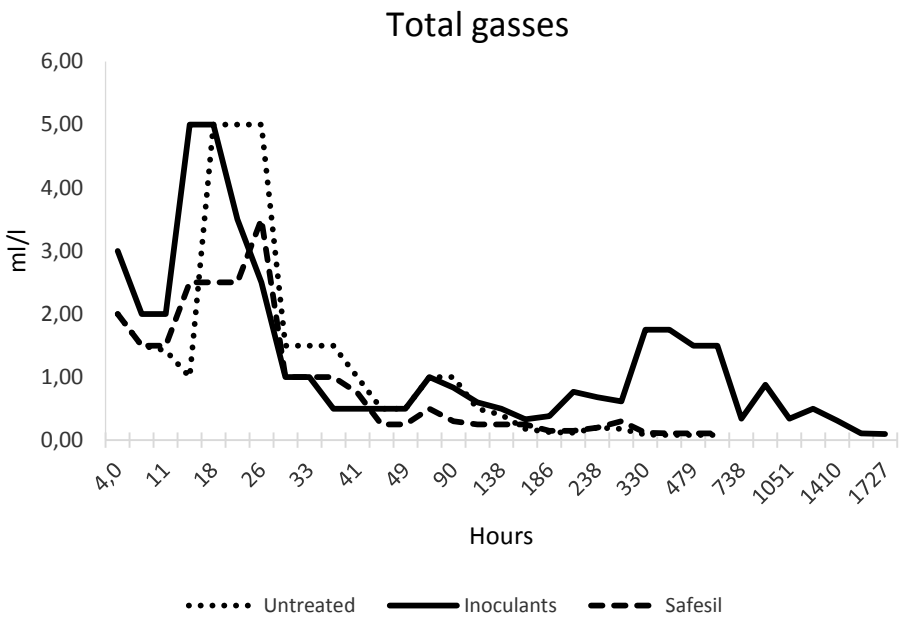

Figure 1. The sum of all gasses formed in silages during measurement. (n=3)

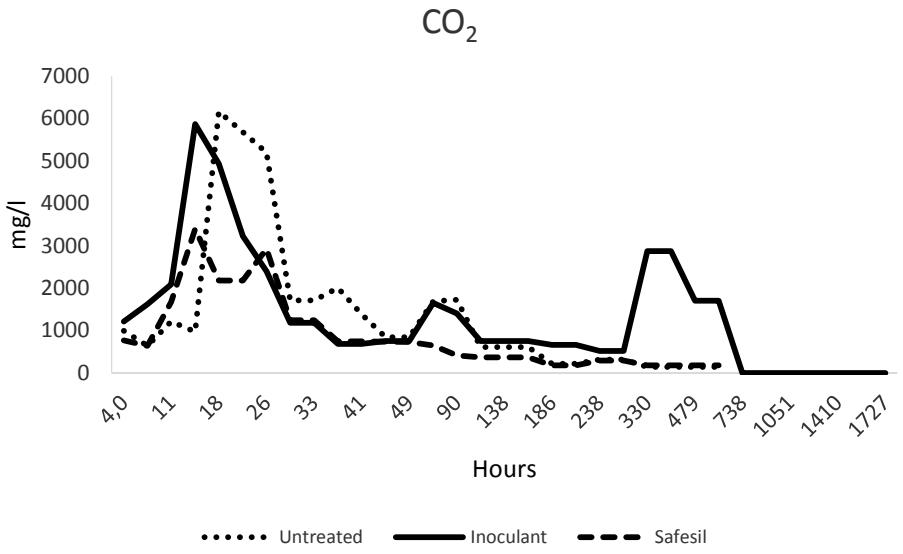

Figure 2. The formation of CO_2 in silages during measurement. (n=3)

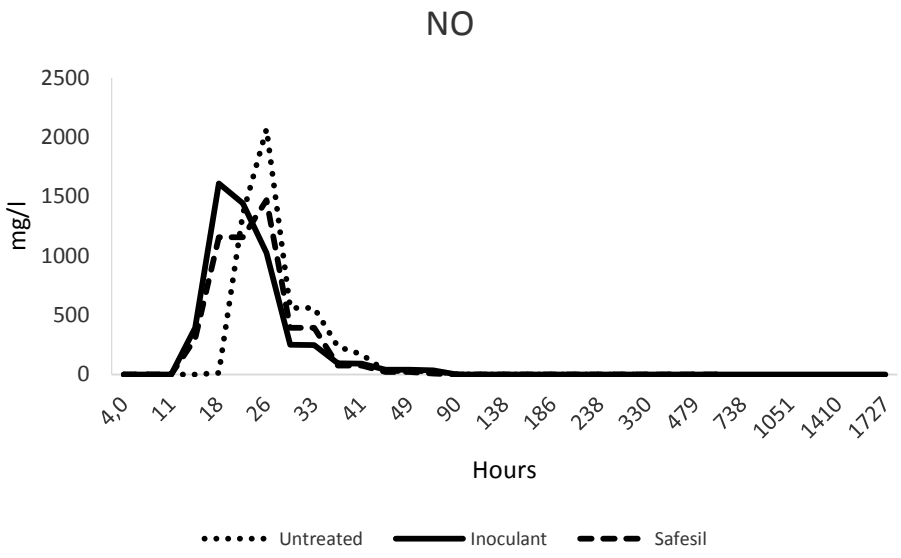

Figure 3. The formation of NO in silages during measurement. (n=3)

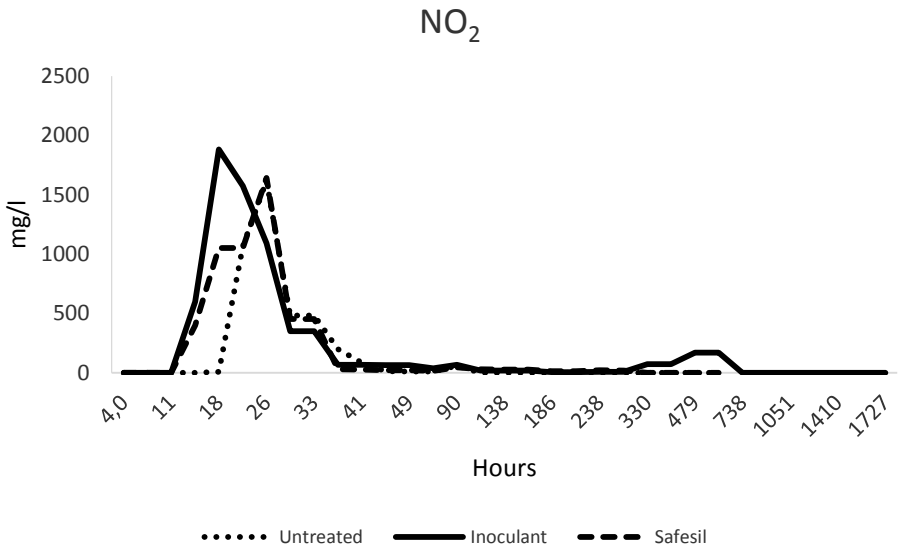

Figure 4. The formation of NO_2 in silages during measurement. (n=3)

Conclusions

All silages were well fermented; however, bacterially inoculated silages contained increased concentrations of acetic and propionic acid and ethanol. Both additive treated silages possessed improved aerobic stability. Control and bacterially inoculated silages produced more gas than Safesil treated silages, mainly due to an increased proportion of CO_2. The formation of NO_x gases displayed no significant differences among other treatments.

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