Effect of silage preparation on methane yields from whole crop maize silages

M. Neureiter, J. Teixeira Pereira dos Santos, C. Perez Lopez, H. Pichler, R. Kirchmayr and R. Braun
Institute for Environmental Biotechnology, Department for Agrobiotechnology, IFA-Tulln, BOKU - University of Natural Resources and Applied Life Sciences, Vienna, Konrad Lorenz Str. 20, A-3430 Tulln, Austria

4th Int. Symposium Anaerobic Digestion of Solid Waste, Copenhagen, Dänemark

Abstract Additives for silage preparation were tested on their effect on the composition of whole crop maize silage and their potential to improve methane production from energy crops in anaerobic digestion plants. Treatments included ensiling without additives, inoculation with silage starters containing homo-fermentative lactic acid bacteria or a mixture of homo- and heterofermentative lactic acid bacteria and the addition of amylase. Additionally the effects of improper silage preparation like insufficient compression of the material and the addition of the spoilage organism *Clostridium tyrobutyricum* were evaluated. Good silage qualities were obtained in all experiments, only the addition of *Clostridium tyrobutyricum* resulted in high concentrations of butyric acid. Methane yields were generally higher after 119 days of storage compared with 44 days of storage. Addition of *C. tyrobutyricum* could significantly improve methane yields based on volatile solids. Methane yields based on freshly harvested material were calculated with respect to the storage losses observed in the experiments. Although *C. tyrobutyricum* caused the highest storage losses, this was compensated by the elevated formation of methane. It remains to be investigated, whether storage losses in laboratory experiments are significant for the technical scale.
Keywords Whole crop maize; silage; lactic acid bacteria; anaerobic digestion

Introduction

Apart from the use of anaerobic digestion as a method for waste treatment, biogas applications focussing on the production of energy based on renewable resources have emerged in the past years. Aims to replace fossil fuels with sustainable energy production systems and the need to fulfill the requirements of the Kyoto Protocol resulted in regulations prescribing quotas for bioenergy and the subsequent promotion of anaerobic digestion for the generation of heat and electricity in several European countries. In Germany and Austria favorable rates for feeding renewable energy into the power grid have led to an increase of biogas plants utilizing energy crops.

Because of its high yields per hectare whole crop maize is the most common substrate for the production of renewable energy by anaerobic digestion. In order to provide continuous operation throughout the year the crops have to be preserved, which is usually done by ensiling. Silage preparation is a well known technology for preserving plant material. Due to compression of the material and air-tight sealing anaerobic conditions are established that promote the growth of autochthonous lactic acid bacteria. The microbial conversion of free soluble carbohydrates into lactic acid and the resulting decrease in pH prevents the growth of undesirable microorganisms (McDonalds et al., 1991).

Microbial silage starters can be added before the ensiling process to ensure or improve silage quality. These additives are defined lactic acid bacteria that can help to control the microbial processes in silage by ensuring a rapid acidification or the formation of specific metabolites that inhibit the growth of spoilage organisms (Weinberg and Muck, 1996; Holzer et al., 2003). Innovations in this field are mainly directed towards the production of high-quality animal feed. However, the use of microbial silage additives also has the potential to improve the economics of biogas plants for bioenergy production. Good silage quality will minimize energy losses during the ensiling process and therefore ensure that most of the energy present at the point of harvesting will be available for the conversion into methane after the storage process. In addition to anaerobic spoilage and the corresponding degradation caused mainly by Clostridia it is necessary to mention the importance of aerobic silage deterioration, which occurs between the opening of the silo and the feeding. Due to the aerobic conditions yeasts and moulds are able to grow and can cause serious energy losses (Honig, 1986; Woolford, 1990). Products that improve the stability of silages are available on the market and can contribute to make the storage process more efficient. Moreover the biochemical processes during ensiling include hydrolysis and acidification. The microbial degradation of crop compounds may lead to a faster conversion or to a better availability of recalcitrant compounds during the anaerobic digestion process so that ensiling can be regarded as a pre-treatment method.

In order to implement an economically successful process for the conversion of energy crops to biogas, it is important to assess the different parameters that can have an impact on methane yields. Methane formation from different types of biomass has been reviewed by Gunaseelan (1997) and influences of varieties and harvesting time have already been described (Amon et al., 2004). However, little is known about the influence of the ensiling process in this context. The aim of this work was to investigate how silage quality and the addition of silage inoculants can affect the methane yield from whole crop maize silage.

Methods

Silage Preparation

Freshly harvested and chopped whole crop maize was used as raw material for all experiments. Silages were prepared in 6.5-liter plastic laboratory silos as described by Danner et al. (2003). In total six different treatments were tested, including a normal silage without additives (control), inoculation with bacterial silage starters S02 and S03 and addition of an amylase preparation. S02 contains homofermentative lactic acid bacteria while S03 contains a mixture of homo- and heterofermentative lactic acid bacteria and is designed to improve the aerobic stability of silage. The silage starters were obtained as freeze dried powder and applied at a concentration of 1 mg/kg of fresh material, which is corresponding to $5 \cdot 10^8$ CFU/kg fresh material. The commercial amylase preparation (Termamyl LC, Novozymes A/S) was applied at a concentration of 450 mg/kg fresh material. Two silages were prepared at conditions that usually will cause spoilage of the material: One of these silages was not compressed and not sealed tightly, which should promote spoilage by aerobically growing microorganisms like yeasts and moulds. The other one was inoculated with *Clostridium tyrobutyricum*, a microorganism that is known to cause anaerobic spoilage of silage by the formation of butyric acid (McDonald et al., 1991). *Clostridium tyrobutyricum* DSMZ 2637 was added from a liquid culture so that the concentration amounted to $7{,}67 \cdot 10^5$ spores per kg of fresh material. To ensure the growth of *Clostridium tyrobutyricum* $CaCO_3$ was added as a buffering agent at a concentration of 15 g/kg of fresh material and the concentration of dry solids was lowered to 27,77 % by the addition of 267 g of water per kg fresh material.

All experiments were carried out in triplicate. The silages were stored at a controlled temperature of 20°C and were opened after 44 days or 110 days, respectively, to perform analyses and anaerobic digestion batch fermentations. Losses during the ensiling process were determined by measuring the weight difference of the silos after preparation and before opening.

Batch Fermentation Tests

Methane production by anaerobic digestion was assessed using a modification of the standard method DIN 38414 S6 (1985). For each silo a batch fermentation test was performed in duplicate. Since three silos were available for each treatment, six tests were made for each ensiling treatment. For the tests 5–6 g of silage were taken and mixed with 350 ml of sludge from a waste water treatment plant. The test reactors were filled to 1000 ml with water. The reactors were incubated at 35°C and continuously stirred with magnetic stirrers. The produced gas was directed through 2 M NaOH in order to absorb the carbon dioxide. The remaining methane was measured by the displacement of slightly acidified water with an indicator solution due to the produced volume of gas. Formation of methane in relation to a blank value (inoculum without substrate) was recorded in regular intervals until no more gas production could be observed. Values were not taken into account when methane production ceased at an early stage of the fermentation. The measured gas volumes were adjusted to standard conditions.

Analytical Methods

Silage was extracted using a laboratory homogenizer as described by Danner et al. (2003). The centrifuged extracts were analyzed for soluble sugars, organic acids and alcohols with a high performance liquid chromatograph (Hewlett-Packard HP1100) equipped with a refractive index detector. For separation of the analytes a Transgenomic ICSep ICE-ION-300 column and the corresponding guard column (Transgenomic ICSep ICE-GC-801/C) was used with 0.01 N H_2SO_4 as solvent at a flow rate of 0.325 ml/min.
The pH-value of the silages was measured with a WTW Microprocessor pH-meter 30 min after homogenizing 20 g sample with 80 ml of water.
Total solids (TS) were determined by drying the material at 105°C for 48 h. Ash content for the calculation of volatile solids (VS) was determined by ashing ground dry samples (2–4 g) at 550°C over a period of 5 h in a muffle furnace.
Ammonia nitrogen and total Kjeldahl nitrogen (TKN) were determined according to Horwitz (1980) using a Gerhardt Vapodest apparatus. TKN was measured from ground samples after disintegration while Ammonia nitrogen was measured from the silage extracts.
Analyses of all samples except for HPLC were performed at least in duplicate.

Results and Discussion

Characterisation of the silages

Soluble carbohydrates in fresh whole crop maize amounted to 23.1 g glucose and 29.3 g fructose per kg TS. Analysis showed that after 44 and 119 days of ensiling all soluble carbohydrates had been metabolized to lactic acid, mannitol, acetic acid and ethanol (Table 1). Butyric acid could only be detected in the silages with *C. tyrobutyricum,* where it constituted the major metabolite after 44 days of ensiling. In all other silages lactic acid was the main product and therefore responsible for the decline in pH to values below 4.0, which enables stable storage. In the silage, that was prepared without compression, sufficient acid formation was observed as well, although the amount of formed metabolites was generally lower in this silage, which is due to the respiration of the carbohydrates to CO_2 at the semiaerobic conditions. Lactic acid formation was unexpectedly high in the silages with *C. tyrobutyricum*. After 119 days a lactic acid concentration of 62.9 g/kg TS was obtained, which was the highest concentration observed. In silages treated with *C. tyrobutyricum* the sum of all formed metabolites was significantly higher than in the other experiments, therefore it seems probable that additional carbon sources were made available by hydrolysis processes.

Table 1 pH and Metabolites in raw material (fresh whole crop maize) and after 44 days (figures in parentheses) and 119 days of ensiling with different treatment methods (n.d.: not detectable)

Treatment	pH	Mannitol	Lactic acid	Acetic acid	Butyric acid	Ethanol
				[g/kg TS]		
Fresh material	5.80	<1.0	<1.0	<1.0	n.d.	<1.0
Control	(3.75) 3.76	(21.7) 19.9	(51.7) 47.9	(16.2) 16.5	(<1.0) <1.0	(13.7) 12.3
S02	(3.74) 3.78	(23.0) 18.3	(52.6) 46.2	(15.0) 16.2	(<1.0) n.d.	(12.9) 13.5
S03	(3.78) 4.09	(11.5) 4.8	(48.1) 17.2	(20.5) 39.2	(<1.0) n.d.	(14.6) 17.2
Amylase	(3.75) 3.74	(21.2) 8.3	(51.3) 45.9	(<1.0) 1.6	(<1.0) <1.0	(13.8) 14.8
Not compressed	(3.89) 3.90	(8.3) 8.9	(44.8) 38.1	(11.1) 14.6	(<1.0) n.d.	(13.3) 4.0
C. tyrobutyricum	(5.00) 4.46	(3.8) n.d.	(33.6) 62.9	(21.9) 20.7	(47.9) 45.0	(21.9) 20.7

Table 2 Weight losses and composition of the raw material (fresh whole crop maize) and silages after 44 days (figures in parentheses) and 119 days of storage

Treatment	Weight losses	TS	VS	TKN	NH_4-N
	[%]	[%]	[%]	[g/kg TS]	[g/kg TS]
Fresh material	-	35.59	34.29	8.6	1.0
Control	(0.81) 0.95	(33.86) 34.92	(32.62) 33.56	(8.6) 8.9	(0.4) 0.6
S02	(0.67) 0.91	(34.18) 34.26	(32.96) 33.08	(10.0) 9.7	(0.5) 0.6
S03	(0.83) 1.28	(32.74) 32.70	(31.38) 31.39	(9.0) 8.6	(0.5) 0.6
Amylase	(0.73) 0.90	(34.23) 33.60	(32.87) 32.32	(9.6) 8.5	(0.5) 0.5
Not compressed	(1.04) 1.34	(34.88) 33.57	(33.65) 32.33	(9.2) 9.3	(0.4) 0.6
C. tyrobutyricum	(1.99) 2.37	(27.74) 27.40	(25.61) 25.31	(9.7) 9.2	(0.7) 0.9

It was expected that addition of amylase would increase the amount of water soluble sugars by hydrolysis of starch and thereby enhance the formation of metabolites. However, such an effect could not be observed. Possibly the low pH-value in the silage inhibited the enzymatic activity. As a consequence further studies should be conducted on whether buffering of silages – as it was done in the treatment with *C. tyrobutyricum* – can improve the activity of externally added enzymes in silage.

Inoculation with additive S03 resulted in a shift from lactic acid to acetic acid between day 44 and day 119. Acetic acid concentrations were highest in these experiments (Table 1). This is due to the activity of *Lactobacillus buchneri*, a component of this silage additive, that is able to convert lactic acid into acetic acid (Holzer et al., 2003). Acetic acid is known to enhance the aerobic stability of silages (Danner et al., 2003) and therefore an improved stability of these silages at the point of feeding the material into the fermenter can be expected. With the exception of the amylase-treated silage, acetic acid was produced in all silages in the range from 15–20 g/kg TS, therefore these silages are expected to show aerobic stability to some extent.

Treatment with homofermentative lactic acid bacteria (S02) did not show significant difference in composition compared with the control silage, however, together with the amylase-treatment the lowest weight losses during ensiling were observed with these experiments (Table 2). As expected, the highest weight losses occurred in the non-optimal silages, i.e. treatment with clostridia (2.37 %) and aerobic spoilage due to improper compression (1.34 %). Elevated losses were also observed with starter S03 after 119 days, which can be explained by the transformation of lactic acid to acetic acid and carbon dioxide by *L. buchneri*, as described by Bucher (1970).

TS, VS, TKN and ammonia-nitrogen as listed in Table 2 are in a comparable range for all the silages and no major changes were observed between 44 and 110 days of ensiling. The exception is the treatment with *C. tyrobutyricum*, where the solids content was artificially lowered during preparation by addition of water.

Methane yields

Table 3 gives an overview on methane yields based on volatile solids, fresh matter, and freshly harvested crop. Treatments that do not differ significantly according to statistical analysis using least significant difference pairwise multiple comparison (LSD) are indicated.

The temporal course of cumulative methane formation from silage samples compared with material before ensiling is depicted in Figure 1. The specific methane yields range between 338 and 537 l/kg VS, which is in most cases higher than reported in literature (Amon, 2004; Gunaseelan, 1997). After 44 days of ensiling the treatments with S03, amylase and *C. tyrobutyricum* show higher specific methane than fresh material, while the other treatments produced similar or slightly lower yields. However, these results can only be regarded as indications since the values do not

Table 3 Maximum methane yields in relation to volatile solids, fresh matter and freshly harvested material with consideration of storage losses. Similar letters in superscript indicate that these values form a homogeneous group when compared with LSD (α=0.05)

Treatment		Methane yields		
		[l/kg VS]	[l/kg FM]	[l/kg freshly harvested maize]
Fresh material		383 [a]	131 [abc]	131 [abc]
Control	(44 d)	338 [a]	110 [a]	109 [a]
	(119 d)	480 [bc]	161 [d]	160 [de]
S02	(44 d)	348 [a]	114 [ab]	113 [ab]
	(119 d)	377 [a]	124 [ab]	123 [ab]
S03	(44 d)	414 [ab]	128 [ab]	127 [abc]
	(119 d)	423 [ab]	133 [abc]	132 [abc]
Amylase	(44 d)	419 [ab]	138 [bc]	137 [bcd]
	(119 d)	486 [bc]	154 [cd]	153 [cde]
Not compressed	(44 d)	355 [a]	120 [ab]	119 [ab]
	(119 d)	405 [ab]	131 [abc]	129 [abc]
C. tyrobutyricum	(44 d)	427 [ab]	110 [a]	138 [bcd]
	(119 d)	537 [c]	136 [bc]	171 [e]

significantly differ at the 5 % confidence level. After 119 days of ensiling higher methane yields than after 44 days were observed in all experiments. Treatment with S02 resulted in slightly lower yields compared with fresh material, while the untreated control silage and the addition of amylase showed elevated methane yields. Treatment with *C. tyrobutyricum* could significantly improve methane formation per kg VS.

Methane yields based on volatile solids can give a good impression for the assessment of silage additives as pre-treatment method. Obviously the addition of *C. tyrobutyricum* leads to the release of compounds that are not available for anaerobic digestion in normal silages. According to chemical analysis a larger amount low molecular weight metabolites are formed in silages with *C. tyrobutyricum*. However, this did not result in a more rapid methane formation, since the methane production curves in Figure 1 run all in parallel. The enhanced methane yields from amylase treatment or the control silage cannot be explained by metabolite formation, so presumably there are intermediate compounds that have not been covered by our analysis.

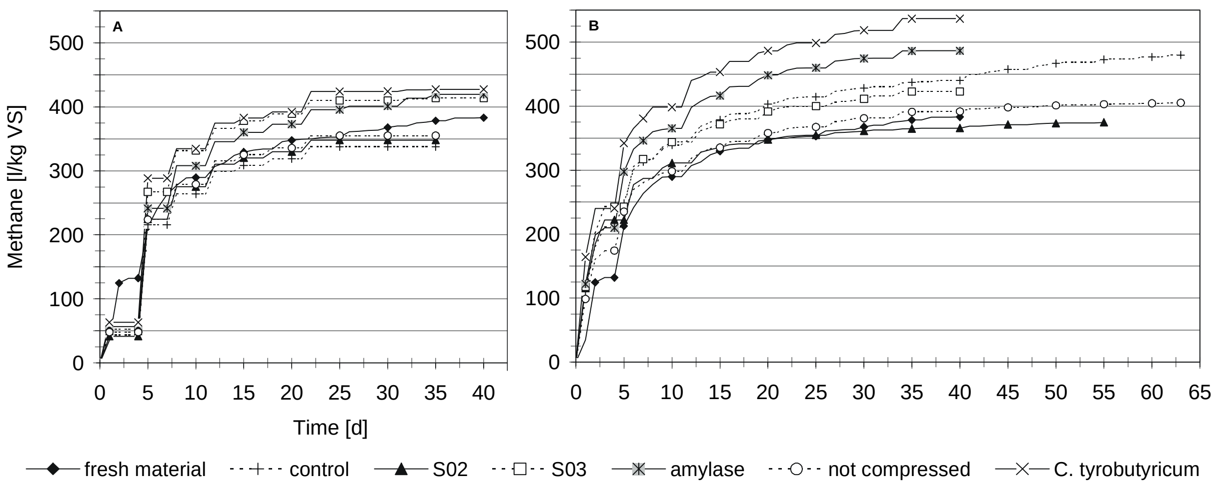

Figure 1 Cumulative methane formation (yields in l/kg VS) from whole crop maize silages after 44 days (A) and 119 days (B) of ensiling.

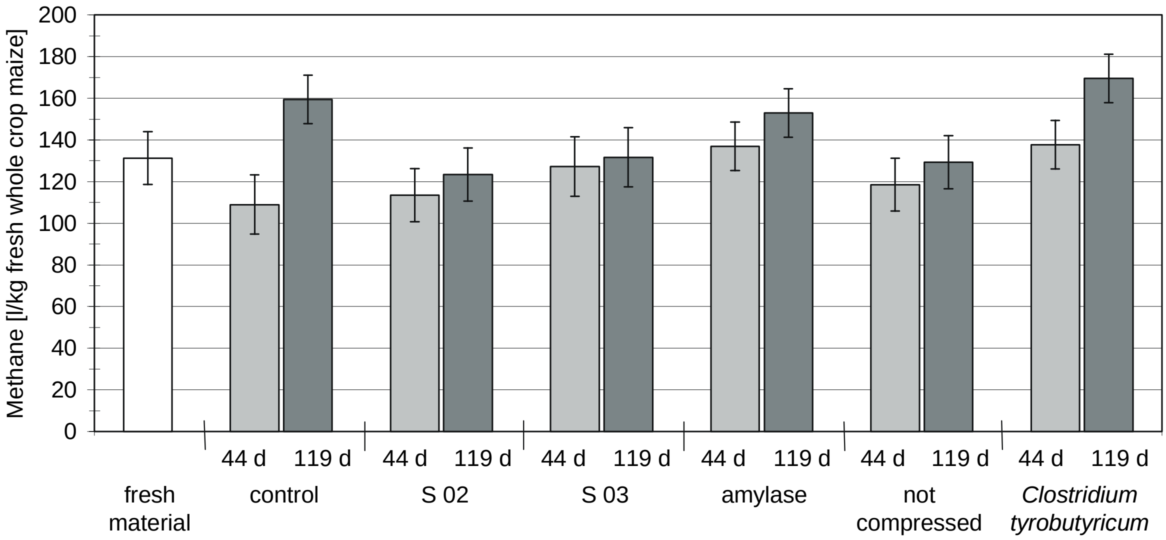

Figure 2 Methane yields of fresh whole crop maize and silages after 44 days and 119 days related to 1 kg of fresh material. Error bars indicate LSD intervals (α=0.05).

For an economical evaluation it is important how much energy can be produced per ton of harvested material after the storage process. Although the biochemical processes may improve the availability of certain components and thus increase methane formation, it is possible that these positive effects are outweighed by energy losses due to microbial deterioration during the ensiling process. Figure 2 displays methane production in relation to fresh whole crop maize with respect to the observed storage losses. Here the most favorable results were obtained after 119 days by the control silage and by the addition of *C. tyrobutyricum*. Remarkably, the treatment with *C. tyrobutyricum* showed the highest losses as well but obviously these losses were compensated by the better availability of the substrate. However, it still has to be proved to what extent losses from laboratory experiments can be related to the situation in technical scale. Furthermore losses due to aerobic deterioration during feeding of continuos reactors could not be considered in these experiments though this might lead to significant losses, especially when large silos are used.

Conclusions

Although ensiling is the method of choice for the preservation of energy crops plant operators often show little awareness that this process affects both energy losses during storage and specific methane yields. The presumption that ensiling generally improves methane yields is not supported by the results, still it seems that longer storage has a favorable effect on the digestibility of the silage.

The results demonstrate that there is a trade-off between good storage properties and maximum methane yields per kg VS. Starter cultures as they are commercially available will improve storage quality but do not enhance methane formation. In fact the treatments with additives S02 and S03 were at the lower range of methane production. In contrast, *Clostridium tyrobutyricum*, a bacterium that is usually unwanted in silage, causes high losses during storage, but these losses are compensated by higher specific methane yields. However, it is difficult to predict the situation in full scale plants, where larger quantities of material must be handled and losses are presumably higher than in labscale tests.

Addition of amylase gave results above the average, but it is doubtful whether this is due to enzymatic activity because it cannot be explained by the chemical composition and the control silage without additives showed similar results. Buffering and water content may play an important role on the activity of enzymes in silages, and may also have contributed to the results with *C. tyrobutyricum*.

Acknowledgements

The authors wish to acknowledge the European Commission for funding this research as part of the CROPGEN (Renewable energy from crops and agrowastes) project (SES6-CT-2004-502824).

References

Amon T., Kryvoruchko V., Amon B., Zollitsch W., Pötsch E. and Mayer K. (2004). Estimation of biogas production from maize and clover grass by the new methane energy value system. In: *Anaerobic Digestion 2004.* Proceedings of the 10th World Congress, August 29 –September 2 2004, Montreal, Canada, pp. 1716–1719

Bucher E. (1970) *Beiträge zur Mikrobiologie der Silagegärung und der Gärfutterstabilität*. Ludwig-Maximilian-Universität München

Danner H., Holzer M., Mayrhuber E. and Braun R. (2003). Acetic acid increases stability of silage under aerobic conditions. *Appl. Environ. Microb.*, **69**(1), 562–567

DIN 38 414 S6 (1985). Bestimmung des Faulverhaltens "Schlamm und Sedimente"

Gunaseelan V.N. (1997). Anaerobic digestion of biomass for methane production. A review. *Biomass and Bioenergy* **13**(1–2), 83-114

Holzer M., Mayrhuber E., Danner H. and Braun R. (2003). The role of *Lactobacillus buchneri* in forage preservation. *Trends Biotechnol.*, **21**(6), 282–287)

Honig H. (1986). Evaluation of aerobic stability. Proceedings of the Eurobac Conference, 12–16 August 1986, Uppsala, Sweden, pp 76–82

Horwitz W. (1980). *Official Methods of Analysis of the Association of Official Agricultural Chemists*, 13th ed., AOAC, Washington D.C.

McDonald P., Henderson A. R. and Heron S. J. E. (1991). *The Biochemistry of Silage*, 2nd edn, Chalcombe Publications, Aberystwyth, pp. 81–151

Weinberg, Z.G. and Muck R.E. (1996). New trends and opportunities in the development and use of inoculants for silage. *FEMS Microbiol. Rev.*, **19**, 53–68

Woolford, M.K., 1990. The detrimental effects of air on silage. *J. Appl. Bacteriol*. **68**, pp. 101–116.