A simple system to study the aerobic determination of silages

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Ashbell, G., Weinberg, Z.G., Azrieli, A., Hen, Y. and Horev, B. 1991. **A simple system to study the aerobic determination of silages.** Can. Agric. Eng. **33**:391-393. A simple laboratory system constructed from 1.5L polyethylene terephthalate (P.E.T.) bottles to measure CO_2 production during aerobic deterioration of silages is described. The aerobic deterioration of corn, ryegrass and wheat silages was studied using the system along with chemical and microbiological analyses. The ryegrass silage was the least stable on aerobic exposure, as indicated by the largest CO_2 production, fastest rise in pH and highest DM losses. Measurement of CO_2 production during aerobic deterioration with the proposed simple system can be a reliable indicator for aerobic spoilage of silages.

Nous avons détaillé un système simple de laboratoire fabrique de bouteilles de polyethylene terephtalate pour mesurer la libération de gaz carbonique au cours de la détérioration aerobique d'ensilage. La détérioration aerobique d'ensilage de maïs, de l'ivraie et de la blé a été etudié en utilisant ce système, et en même temps des analyses chimiques et microbiologiques. La plus grand libération de gaz carbonique, la plus rapide augmentation en pH, et des pertes de matière sèche les plus elevées ont tous indiqués que l'ensilage d'ivraie a été le moins stable après exposition à l'air. Mesurer la libération de gaz carbonique pendant la détérioration aerobique en utilisant ce système simple pourrait fournir un indicateur fiable pour la détérioration aerobique d'ensilage.

INTRODUCTION

Air (oxygen) is a major cause of spoilage of silage because it enables undesirable chemical and microbiological activities to occur which result in silage deterioration (McDonald 1981; Woolford 1990). Air can penetrate into silage during silo filling, storage, and the feed-out period. The deterioration is accompanied by a rise in temperature which is directly related to oxidative dry matter (DM) losses in the form of carbon dioxide (Woolford et al. 1977). The rate of CO_2 production is also an indicator for the intensity of aerobic spoilage of silage and DM loss.

Various systems have been used to study aerobic deterioration of silages. Henderson et al. (1979) used small polystyrene containers covered with loose lids. Ohyama et al. (1977) described a similar system. These authors measured temperature and pH rise and additional chemical and microbiological parameters during the exposure of the silages to air. Woolford et al. (1977) developed a laboratory scale system to measure the aerobic deterioration of silages. In that system a relationship was established among DM losses, temperature rise and CO_2 production. Those authors used this technique to study the chemical and microbiological changes that occur during aerobic deterioration of silage (Woolford et al. 1978, 1979).

The objective of the present work was to test a simple system constructed from polyethylene terephthalate (P.E.T.) bottles to study the aerobic deterioration of silages in laboratory experiments.

MATERIALS AND METHODS

Unit description

The unit constructed from 1.5L P.E.T. (polyethylene terephthalate) bottles is shown in Fig. 1. Polyethylene terephthalate is a stable, corrosion-resistant, gas-tight material, used in the carbonated soft drink industry and has a CO_2 transmission rate of 15-25 ml/mil/100 square inch/24 h at 1 atmosphere and 25°C (Modern Plastics Encyclopedia 1981). To prepare one test unit, two bottles were used; the upper part of one bottle was cut to 1 L volume to serve as the upper part of the system. The original base of the bottle served as the lid for that part. Two 1-cm diameter holes were bored, one on the top and one on the bottom of the upper part to enable air circulation. The holes were protected by nets against insects. Silage was

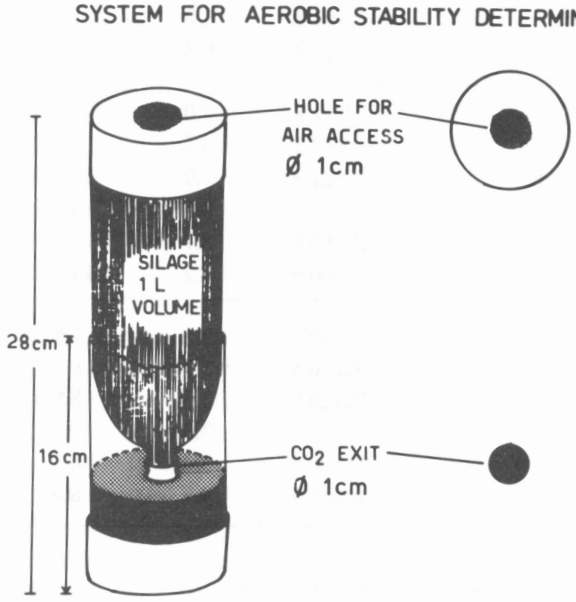

Fig.1. System for aerobic stability determination.

loosely packed in this part (250-300 g on wet basis). The lower part of the unit, which was made from another bottle, was filled with 100 ml of 20% KOH. The upper and the lower parts fit together and formed the system (Fig. 1).

The aerobic activity which took place in the silage samples resulted in production of CO_2, a gas which is 1.5 times as dense as air, and therefore sinks to the bottom and is absorbed by the base (which is in excess). The solution was titrated with 1N HCl which expelled the CO_2. The amount of CO_2 (g/kg DM) was calculated according to:

$$CO_2 = 0.044\ T \cdot V / (A \cdot FM \cdot DM)$$

where:

T = volume 1N HCl used in titration (ml),
V = total volume 20% KOH (ml),
A = volume KOH used in determination (ml),
FM = mass of fresh material (kg), and
DM = fraction of dry matter.

Experimental procedure

Corn, ryegrass and wheat silages of good quality taken from a freshly exposed face, immediately after unloading of commercial bunker silos were tested in these units for aerobic deterioration. The units containing 300 g wet silage were stored at a temperature of 21±2°C. The CO_2 produced was determined as described above, and the silages were also subjected to chemical and microbiological analyses. There were 15 test bottles per forage, three bottles of which were tested on days 2, 4 ,6, 8 and 10 of the experiment.

Chemical analysis

DM was determined by oven drying at 105°C for 24h.

Ash content was determined by heating the dry samples in an oven at 600°C for 2h.

pH was measured on the filtrate of 10g wet material blended for 5 min in a Stomacher blender with 90 ml distilled water.

Lactic and volatile fatty acids were determined by HPLC, using a column packed with C-3 cation exchange resin (Technicon, Chauncey, NY).

Microbiological examinations included the enumeration of lactic acid bacteria (LAB), yeasts (Y), lactic-assimilating yeasts (LY), molds (M), enterobacteria (E) and clostridial spores (CL). The methods employed have been described fully by Ashbell et al. (1987).

RESULTS AND DISCUSSION

The chemical and microbiological analyses of the three silages that were exposed to air are summarized in Tables I and II, respectively. Deterioration of silage under aerobic exposure was accompanied by a rise in pH, DM losses and an increase in ash content. Deterioration were also accompanied by rapid growth of yeasts and molds which was uniformly distributed in all parts of the bottle. This indicates that there was a free flow of air through the whole volume of the system. Most of the yeasts were lactate-assimilating yeasts and this might explain the rise in pH and loss of lactic acid during aerobic exposure. The rate of aerobic spoilage depended on the chemical and microbiological compositions of the silage. The corn silage was the most stable upon aerobic exposure. This was

Table I. Chemical analysis of the silages exposed to air

Forage	Time (days)	%DM	pH	Ash[1]	Latic[1] acid	Acetic[1] acid	Ethanol[1]	%DM[2] loss	CO_2 production (g/kg DM)
Corn	0	42.9±0.9	3.9	4.5	2.8	0.6	5.2		
	2	43.5±1.3	4.0	4.6	2.2	0.5	4.9	2.4±0.7	0.9±0.2
	4	42.8±0.7	4.4	4.6	1.8	NF	1.7	0.4±0.4	10.5±1.5
	6	43.2±1.2	5.4	4.6	0.5	NF	NF	2.0±1.1	16.3±0.7
	8	41.8±1.8	6.0	4.8	-	-	-	3.8±4.0	18.7±1.5
	10	42.8±1.7	6.8	4.8	NF	NF	NF	4.0±3.0	21.4±0.9
Ryegrass	0	52.9±1.8	5.0	12.4	1.0	0.5	NF		
	2	51.3±2.8	6.5	13.0	0.7	NF	NF	3.8±5.2	15.1±3.9
	4	52.8±2.3	7.9	13.7	-	NF	NF	2.8±3.4	31.2±5.6
	6	55.0±2.7	8.5	14.4	NF	NF	NF	3.2±0.8	37.6±4.5
	8	52.3±0.3	8.7	13.9	NF	NF	NF	3.5±0.5	45.8±2.4
	10	48.0±0.9	8.2	13.7	-	NF	NF	11.9±1.8	139.2±25.0
Wheat	0	29.3±0.7	3.1	7.5	-	-	-		
	2	28.9±0.5	3.3	8.0	-	-	-	1.6±1.5	5.4±1.5
	4	27.6±0.6	3.3	8.5	15.1	1.8	3.3	6.2±1.8	6.9±1.2
	6	27.2±0.2	3.3	8.4	8.6	4.5	NF	7.8±0.6	29.3±4.5
	8	28.4±0.2	3.4	8.3	5.8	4.9	NF	4.0±0.6	39.6±3.4
	10	26.6±1.1	4.7	8.6	3.9	1.7	NF	11.1±4.0	78.3±21.9

1 - percent in DM
2 - calculated by mass and %DM differences
NF = Not found

Table II. Microbial analysis of the silage exposed to air (log number of colony forming units per g DM)

Forage	Time (days)	Lactobacilli	Yeasts	Lacate assimilating yeasts	Molds	Enterobacteria	Clostridial spores
Corn	0	7.4	3.4	3.4	2.4	NF	1.5
	2	7.2	7.7	7.7	6.0	NF	2.4
	4	7.7	8.8	8.5	9.0	NF	1.5
	6	8.0	9.6	9.3	9.1	NF	NF
	8	7.8	9.1	8.5	9.2	NF	0.8
	10	7.9	9.3	9.0	9.4	NF	1.4
Ryegrass	0	8.7	8.3	7.3	7.9	6.9	3.5
	2	9.8	9.8	8.9	9.4	7.7	2.4
	4	10.0	9.2	9.4	9.3	8.7	2.9
	6	9.9	9.5	8.9	9.2	9.2	2.5
	8	10.1	9.9	9.1	9.2	8.9	2.2
	10	9.8	9.4	9.1	9.1	9.6	2.6
Wheat	0	4.5	5.7	2.6	NF	NF	NF
	2	6.1	7.7	3.3	NF	NF	NF
	4	7.3	8.0	3.7	NF	NF	1.4
	6	7.9	9.1	5.6	NF	NF	1.2
	8	7.3	9.1	4.5	5.1	NF	1.4
	10	8.1	9.7	4.1	5.2	1.3	2.0

NF = Not found

indicated by a slower rise in pH, less CO_2 production and smaller DM losses. The ryegrass silage deteriorated very quickly. The pH of this silage on day 0 was higher than the pH of the wheat and corn silages, and this also enabled the enterobacteria to be present in large numbers.

Correlation coefficients between CO_2 production and pH change during aerobic exposure for the three silages were high (0.99, 0.85 and 0.82 for corn, ryegrass and wheat, respectively). Correlation coefficients between CO_2 production and DM losses during aerobic exposure were significant ($P>0.05$) for the ryegrass and wheat silages (0.72 and 0.73, respectively). For the corn silage, DM losses were low, as was the correlation coefficient (0.42). This low correlation coefficient might be explained by the fact that determination of silage DM losses by difference according to oven-drying might be overestimated, due to the loss of volatile fatty acids. This was true especially when DM losses were small. The proposed method enables to estimate DM losses more accurately by multiplying the amount of CO_2 by a factor of 0.68. In their studies of aerobic deterioration of silages, Henderson et al. (1979) also suggested the use of other parameters (pH and temperature rise) rather than DM losses as indicators for aerobic spoilage. However, temperature measurements require insolated systems and this would have made the proposed system less simple.

The proposed system prepared from P.E.T. bottles is cheap, easy to set up and to standardize, and samples can be withdrawn at different intervals. The measurement of CO_2 production, separately or in combination with other measurements (such as pH, lactic acid and volatile fatty acids, microbial examination), can serve as a reliable method to determine the aerobic deterioration of silages in the laboratory.

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