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A laboratory silo permitting repeated sampling

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Summary

A new type of laboratory silo for studying silage fermentation is described. Samples from the silo are taken manually by use of a glove which is mounted on the silo. Introduction of air during sampling is prevented by placing the sample in a sample lock.

In a preliminary experiment the accumulation and disappearance of nitrite in wilted grass ensiled in the laboratory silo was monitored. The results showed less variation than when the same grass was ensiled in preserving glasses.

Introduction

Laboratory silos have been used widely for the investigation of silage fermentation and related processes. These silos have capacities ranging from tens of grams to a few kilograms and must be sampled repeatedly when information about the advancement of silage fermentation is wanted. Usually, sampling is not possible without the introduction of air, which disturbs the fermentation.

This problem can be overcome by incubating a number of replicates and by considering the contents of one silo as a sample (McDonald, 1981). This approach has proven its usefulness when chemical parameters of the silage process are studied and the intervals between successive samples are in the order of at least some days. Whenever fast processes are to be studied, as the development of the microbial flora, such an experimental design is inadequate, because it cannot be assumed that the fermentations in the different silos proceed synchronized.

From the foregoing considerations the laboratory silo to be described here was developed. The silo permits repeated sampling without disturbing the gas phase.

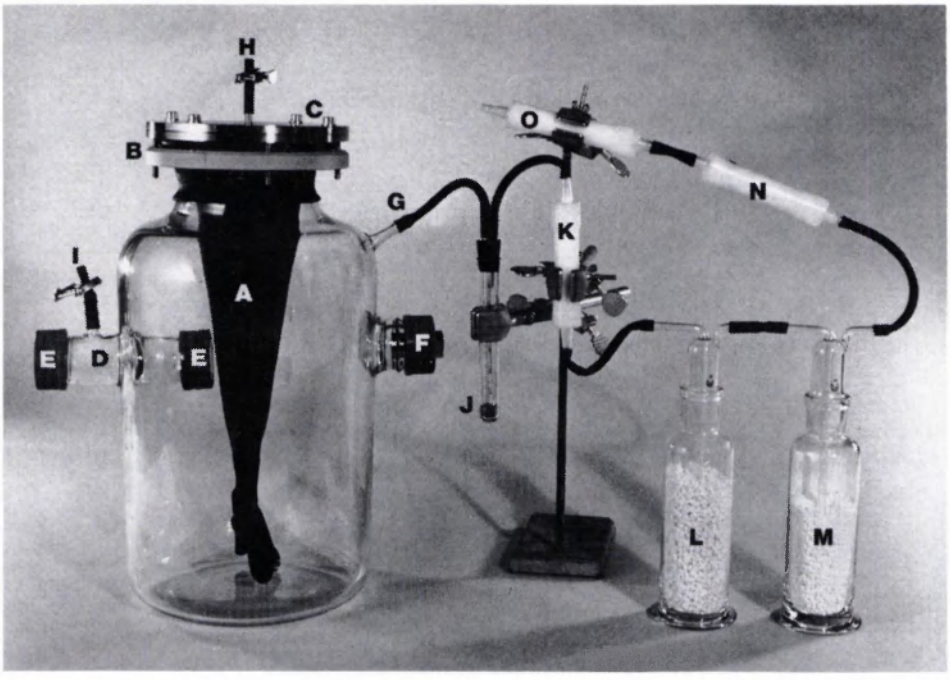

Fig. 1. The laboratory silo with CO_2-absorption train.
A, Butyl-rubber glove; B, metal flange; C, stainless steel lid; D, sample lock; E, serum bottle screw caps; F, gas sampling port; G, gas outlet; H and I, vents; J, mercury valve; K, moisture trap ($CaCl_2$); L, CO_2 trap (soda lime); M, moisture trap ($CaCl_2$); N, moisture trap ($CaCl_2$); O, CO_2 trap (soda lime).

The laboratory silo

The silo (Fig. 1) was constructed by Applikon B.V. (Schiedam, Netherlands) according to our specifications. It is built from a 10-litre pyrex flask with a flat neck and an opening with a diameter of 10 cm. A butyl rubber glove (length about 50 cm) is folded over the neck of the flask and held in place by a metal flange. Most commercial rubber gloves contain secondary amines, which react with the nitrite formed during fermentation to nitrosamines. Health risks caused by these carcinogenic compounds to the person that samples the silo can be avoided by using nitrogen-free butyl-rubber gloves (Norton Company, Charleston, USA). Gas-tight sealing of the glove is achieved by rubber O-rings in the upper and lower part of the flange facing the glove. On top of the flange a stainless steel lid is fastened with 4 screws. The lid also seals gas-tightly and prevents diffusing of gases through the glove into the silo.

A glass tube (length 10 cm, outer diameter 4 cm) with screw thread at both ends is constructed through the sidewall of the flask. The tube is closed by serum

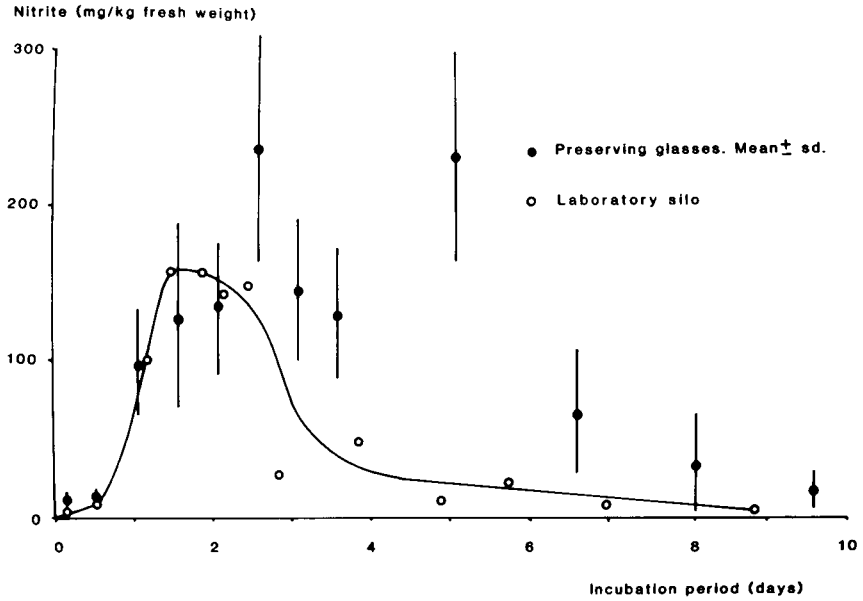

Fig. 2. Accumulation and disappearance of nitrite in grass ensiled in the laboratory silo and in preserving glasses.

bottle screw caps (type Schott GL 45) and serves as a sample lock (volume 138 ml).

In the sidewall opposite to the sample lock a gas sampling port is made. It consists of a stainless steel septum holder mounted in a serum bottle screw cap that fits on a glass tube in the silo wall. A gas outlet is made above the gas sampling port. The pressure in the silo is kept at 0.4 cm mercury column by a simple mercury valve. Excess gas is led through a CO_2-absorption train. It consists of two flasks, one filled with soda lime (L) and one filled with $CaCl_2$ (M). A smaller tube (K) with $CaCl_2$ is placed between the mercury valve and the absorption train to absorb moisture from the gas. Behind the train two tubes with $CaCl_2$ and soda lime, respectively, are placed to avoid absorption of CO_2 and moisture from the air. By weighing the absorption train periodically CO_2 production is measured.

The silo is filled with grass through the top opening up to the level of the sample lock (capacity 2-3 kg). Afterwards the glove, flange and lid are put into place again. Silage samples are taken by the gloved hand after removing the lid and the screw cap at the inner side of the lock. The sample is placed in the lock, which is then closed again. After sampling the lid is fastened and the volume of the glove minimized by applying vacuum through vent H. During the sampling procedure the gas outlet is closed. The sample is removed from the outside of the lock. To avoid introduction of air in the silage the lock is evacuated or flushed

with an inert gas through vent I. Gas samples are drawn with a gas-tight syringe from the gas sampling port and analysed gas-chromatographically.

Application

The laboratory silo was developed primarily for studying the influence of various parameters in silage fermentation on the development of lactate-fermenting clostridia.

A preliminary experiment was performed in which the temporal accumulation of nitrite, a well-known inhibitor of clostridia, was monitored. Wilted perennial ryegrass (27 % dry matter (dm), 10 % crude protein in dm, 1 % nitrate in dm and 8.0 % total sugar in dm) was ensiled in the laboratory silo and in 2-litre preserving glasses and stored at 30 °C. Periodically a sample of 30 g from the laboratory silo and 5 replicate preserving glasses were taken for analyses of nitrite.

Rapid acidification occurred (pH 4.3 after 5 days) and no clostridial activity took place in the laboratory silo. A few hours after ensiling nitrite was already present the silages. In the laboratory silo the maximum concentration of nitrite (156 mg/kg fresh weight) was measured two days after ensiling. The maximum in the preserving glasses amounted to 220 mg/kg fresh weight (average of five replicates) and was measured on the third and fifth day. These values seem, however, to deviate from the nitrite levels found in the other preserving glasses. In general the variation in nitrite concentrations of replicates was large, indicating considerable differences between individual fermentations. From the third day the nitrite levels in the laboratory silo were lower than the mean values in the preserving glasses. After ten days only minor concentrations of nitrite were left in the silages.

The obtained results indicate that the laboratory silo offers advantages when the fast microbial and chemical processes that occur during the first days of silages fermentation and their interactions are studied.

Acknowledgement

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Reference

McDonald, P., 1981. The biochemistry of silage. John Wiley & Sons, Chichester, pp. 14-19.