A novel method to simultaneous determination of methane production during in vitro gas production using fully automated equipment

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Introduction

The automated gas production equipment, as described by Cone et al. (1996) uses electric valves to vent the system, to prevent overpressure in the bottles and there was no possibility to analyze the composition of the vented gas. The gas production equipment, was adapted so that small quantities (10 μL) of gas can be taken from the headspace, to be analyzed for methane concentration. The aim of this study was to investigate the possibilities of this adapted gas production system to accurately measure methane synthesis kinetics and to calculate the methane/total gas production ratio without disturbing the overall gas production measurements. To proof this, gas production and methane concentration in the produced gas was determined for feedstuffs without and with some selected additives, known to influence methane production.

Material and Methods

An adapted version of the gas production equipment, as described by Cone et al. (1996), was used. The bottles were adapted and fitted with a glass extension and sealed with a screw cap and an air-tight septum. Using a gas tight syringe small aliquots of 10 μL were sampled sequentially from the headspace gas and analyzed for methane.
Gas production incubations were performed as described by Cone et al. (1996). Rumen fluid was collected from two lactating rumen cannulated Holstein Friesian cows, 2 h after the morning feeding. Each experiment was done in a separate single run. Gas production measurements were corrected for blank gas productions (i.e., gas productions in buffered rumen fluid without sample).
To test the accuracy of the determination of methane production, a bottle (total volume 300 ml) with 60 ml buffer in a water bath of 39°C was filled with pure CO_2 and 10 times 10 ml of pure methane was injected via a syringe through a special tap fitted on the screw cap. The injection of 10 ml of methane resulted in the venting of 10 ml of gas from the head space by the venting system. After the methane injection, 10 μl of the gas in the headspace was obtained via the glass capped extension using the gas tight syringe and analyzed for methane. The whole procedure of 10 injections with 10 ml of methane was repeated 5 times after which the entire procedure was repeated again 5 times with injections of 10 ml of methane followed by 40 ml of CO_2. The determined methane concentrations in the headspace were transformed to amounts of "synthesized" methane, taking account of the composition of the vented gas and dilution with the gas in the headspace.
Duplicate incubations of 0.5 g maize and soybean hulls were conducted with different amounts of additives reported to inhibit methane synthesis: sodium-2-bromoethanesulphonate (BES, 15 mg/0.5 g OM incubated), monensin sodium salt (15 mg/0.5 g OM), cinnemaldehyde (150 mg/0.5 g OM) and tea tannins (150 mg/0.5 g OM). Total gas production was recorded as a measure for the organic matter fermentation and after different time intervals methane concentration in the headspace was determined and synthesized methane by the fermentation was calculated taking account of the vented gas and dilution with the gas in the headspace. Methane was determined using a gas chromatograph fitted to a flame ionization detector, using a packed column (PorapakQ, 6 mx1/8 inch, 50-80 mesh, Grace/ Alltech, Lexington, Kentucky, USA) with nitrogen as carrier gas and an oven temperature maintained at 60°C.

Results and Discussion

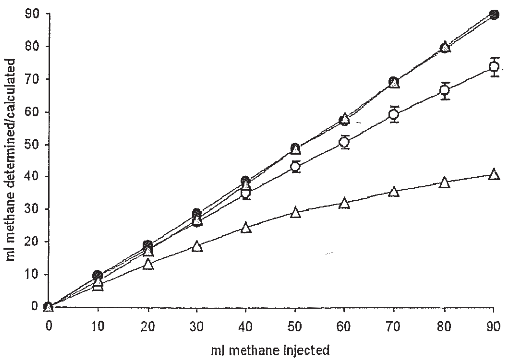

Fig. 1. Relationship between injected and determined volume of methane in the headspace of the bottle (O, Δ) and the calculated total volume of methane injected, taking into account the vented gas (▲, ●). The headspace was filled with CO2 and methane in the headspace was determined after each injection of 10 ml methane (O, ●) or 10 ml methane plus 40 ml air (Δ, ▲) in the venting system, releasing the same amount of gas as injected.

Figure 1 shows that the determined methane concentrations in the headspace can be transformed into actual "synthesized" methane during fermentation experiments, taking account of the concentration of the vented gas and the dilution with the gas in the headspace.

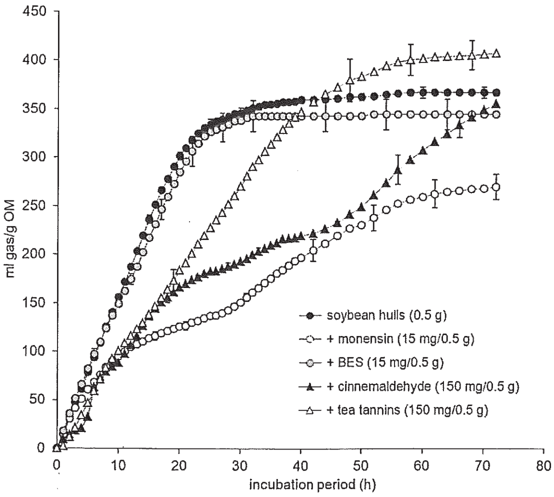

Fig. 2. Gas production profiles of a soybean hull sample with different additives (dose/0.5 g of soybean hulls).

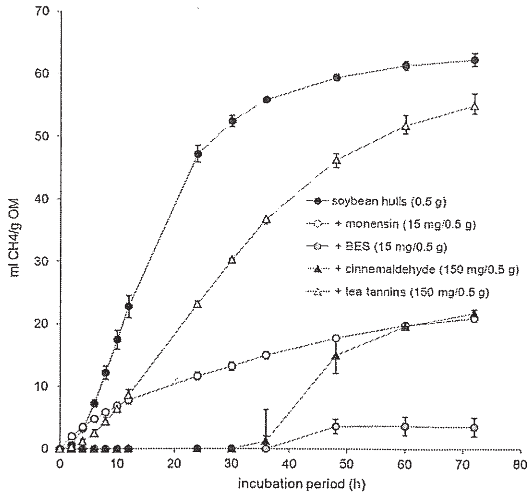

Fig. 3. Methane (CH4) production profiles of a soybean hull sample with different additives (dose/0.5 g of soybean hulls).

Figure 2 shows the total gas production of a sample soybean meal, without and with additives, known to influence methane fermentation. Figure 3 shows the methane synthesis during fermentation of soybean meal, without and with the additives. The results clearly show that all additives had an effect on the rate and extent of gas production of the soybean hulls, with the exception of BES (Fig. 2). The additives monensin, cinnamaldehyde and tea tannins decreased the rate of gas production. All additives had an influence on methane synthesis (Fig. 3). The results show that the adapted gas production equipment offers the possibility to measure organic matter fermentation (= gas production) and methane synthesis simultaneously. With this system the maximum level of fermentation and methane synthesis can be determined, as well as the kinetics of synthesis. With this technique a fast screening of feedstuffs and additives for methane synthesis and OM fermentation in vitro is possible.

References

Cone, J.W., Van Gelder, A.H., Visscher, G.J.W., Oudshoorn, L., 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. Anim. Feed Sci. Technol. 61, 113-128.

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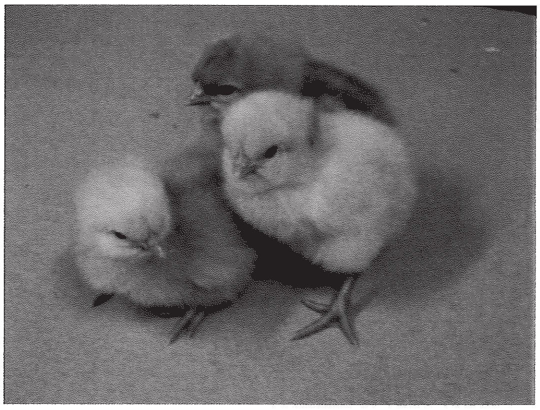

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