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A fully automated incubation system for the measurement of gas production and gas composition

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ABSTRACT

A fully automated *in vitro* incubation system consisting of 32 incubation bottles (125 ml) each fitted with a pressure sensor and a solenoid valve was developed to monitor total gas production and hydrogen (H_2) and methane (CH_4) composition. Gas volume is calculated from the pressure readings collected by a dedicated computer and when the threshold pressure of 9 kPa is reached the fermentation gases are released into a gas chromatograph *via* a sampling loop. Substrates were incubated at 10 mg/ml in 60 ml of a medium with a buffer to rumen fluid ratio of 4:1. Typically, 20–25 measurements of gas volume and composition are obtained from each bottle over a 48 h incubation period. More than 60% of the measurements are obtained during the first 12 h of fermentation when rumen fermentation is most active. This allows the same model to be fitted to the data to describe the gas and CH_4 production data and directly compare their kinetics for various substrates. The overall repeatability of the system has been tested over 2 years using a 'standard ryegrass hay' sample that was incubated as an internal control in each incubation run. Overall, repeatability of gas and CH_4 production was comparable to other incubation systems with a CV of 7.2% and 12.5% for total gas and CH_4 production. However, within the dataset an annual pattern was detected in the parameters of the standard hay. Differences in CH_4 emissions from various substrates were largely explained by differences in short chain fatty acid production.

In an initial series of test the system was used to evaluate the effect of incubation buffers or rumen fluid donor species on gas emissions and fermentation end products. While no differences were found for the different buffers used, our results indicate sheep rumen fluid leads to a slightly lower production of CH_4 when compared to rumen fluid from cattle.

The system presented here is the first rumen fluid batch culture system that automatically measures total gas, CH_4 and H_2 production and is currently used as a screening tool for substrates or additives that have a potential to lower CH_4 emissions from ruminants.

1. Introduction

In vitro rumen incubation systems have been used for decades to rank ruminant feeds according to their potential feeding value, to supplement data derived from wet chemistry analysis of these feeds. Early *in vitro* systems focused on endpoint

Abbreviations: DM, dry matter; ADF, acid detergent fibre; aNDF, amylase treated neutral detergent fibre; GC, gas chromatograph; SCFA, short chain fatty acids; TCD, thermal conductivity detector; FID, flame ionisation detector.

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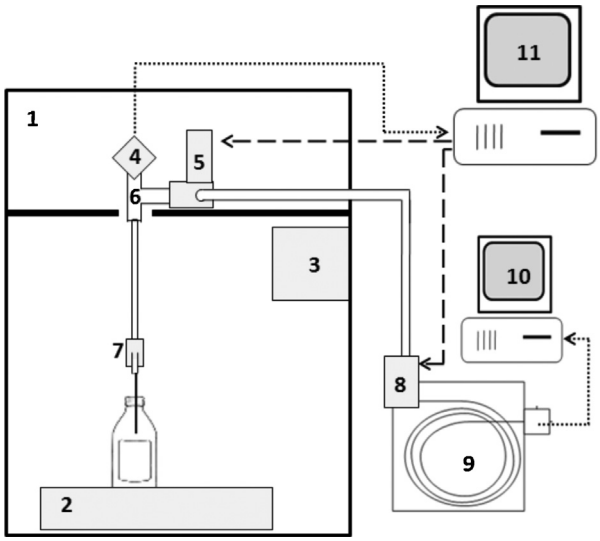

Fig. 1. Components of the incubations system: 1, incubator; 2, reciprocal shaker; 3, fan; 4, pressure sensor; 5, 3 way valve; 6, T-piece; 7, Luer extension and needle; 8, 6-port sampling loop; 9, gas chromatograph; 10, operating computer; 11 monitoring computer. Dotted lines indicate signal inputs from sensors and detectors; dashed lines indicate output signals to valves.

measurements such as substrate degradability (Tilley and Terry, 1963). In the 1970s it was recognised that accumulation of fermentation gases in combination with chemical composition data of the substrates can be used to estimate the metabolisable energy content and the degradability of the organic matter from ruminant feeds using the Hohenheim gas test (Menke et al., 1979). This system uses glass syringes as incubation containers and gas volume is determined from the position of the piston that moves up as fermentation gas is produced. This gas-based system was a step forward since the measurement did not destroy the sample and gas readings could be taken repeatedly in order to determine the rate of fermentation. In the 1990s the first automated pressure based systems were developed (Pell and Schofield, 1993) providing real-time measurements of gas accumulation allowing for a better understanding of the kinetics of fermentation of different plant species (Groot et al., 1996; France et al., 2005). The first automated systems accumulated the fermentation gases over a whole 24-h period. Later it was recognised that the increased pressure within the fermentation container can affect fermentation rate and extent (Tagliapietra et al., 2010) and fermentation end products (Jouany and Lassalas, 2002). More recent systems periodically release the pressure *via* a solenoid valve (Cone et al., 1996; Davies et al., 2000) thus avoiding the build-up of pressure. Analysis of gas composition for these systems still involves manual sample collection and injection into a gas chromatograph (Martínez et al., 2010; Pellikaan et al., 2011).

This paper describes a further development of an automated gas measurement system where the gas production is monitored in real-time *via* pressure sensors, and the proportion of CH_4 and H_2 in the vented fermentation gases is measured automatically in a gas chromatograph. This system allows accurate measurement of the rate and amount of individual gases produced. In order to assess the repeatability of the gas and CH_4 measurements between incubations, data from a standard feed used in every incubation have been evaluated. In addition, the response of the system to different *in vitro* incubation buffers and rumen fluid donor species was examined.

2. Materials and methods

2.1. Description of the system

The system consisted of 4 arrays of eight 100-ml serum bottles located on a reciprocal shaker set to 90 rpm in a fan-driven 39 °C incubator. Two-port solenoid driven valves (Model V114A-5LOU, SMC, Auckland, NZ) immediately downstream of the bottle's pressure sensor were located on a base plate above the bottles. The pressure sensor (Model 393-8339, RS components, Auckland, NZ) was connected to the bottle *via* an extension tube (3.2 mm OD) and Luer lok® fitting connected to a 23 g needle inserted through a butyl rubber stopper of the bottle (see Fig. 1 for valve and fitting details). To limit the dead volume between the 3 way valve and sample injection loop of the GC, the 3 way valves are connected in series in 4 arrays of 8 valves. The output from each array was then attached to a set of four additional 3 way valves (array valves) in series (see Fig. 2). When a bottle's 3 way valve and its associated array valve were not actuated (normal closed position), fermentation gas accumulated in the bottle and the tubing downstream of the bottle's 3 way valve is continuously flushed and vented with N_2 gas. When a bottle's 3 way valve and its associated 3 way array valve were actuated (opened position), the N_2 flushing gas is shut off and a bottle's accumulated gas was vented through a 20 μL gas sample injection loop attached to a 6 port rotary valve (Valco GC, VICI, Texas, USA) and gas chromatograph (GC) column. After 15 s of venting the bottle's valve closed and the GC sample injection