The new Gas Endeavour system from Bioprocess Control AB for *in vitro* assessment of animal feeds

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Introduction

In vitro rumen incubation analyses have already been used for years to evaluate the nutritive qualities of feeds, originally employing end-point measurements focusing on feedstuff digestion. The relation between accumulation of fermentation gases and metabolisable energy content of the feed was established in the 1970s. Since then, measurement techniques based on *in vitro* gas production have been further developed for feed evaluation experiments (Cone et al., 1996; Getachew et al., 1998; Getachew et al., 2008; Murray et al., 2014). Much of these early reports rely on a manometric gas measurement principle. Not many publications on liquid displacement systems based on a volumetric gas measuring principle are available due to the limitations of instrumentation setup.

This work describes a new volumetric gas measuring technique specially developed for monitoring production of ultra-low gas volumes, with various applications in batch fermentation tests. The technique has been successfully applied and validated for quantifying biochemical methane potential from various biodegradable organic matters. An automated measuring system based on this volumetric measuring technique can offer continuous monitoring of gas production from *in vitro* digestibility tests with high throughput and significant reduction of labour and time intensity.

Background

There are many protocols available on how to perform *in vitro* digestibility tests. Some of them are adapted for utilisation of the gas measurement technique, but they differ in experimental set up and are generally modified and adapted to the specific researcher´s purpose. Because of this, it is often difficult to evaluate results from different studies and values can vary substantially. Thus, there is a need for a test standard and general procedure, but also for a measurement quality standard of the gas measurement technique for *in vitro* digestibility tests.

One issue that is not fully addressed in the current protocols, is the equipment and experimental set up that is used for these kinds of tests. Many times, these are developed in-house and specific for each laboratory. A solution to minimise differences is the use of a complete lab platform such as the Gas Endeavour (Figure 1 and 2). The Gas Endeavour is specially designed for low gas volume and flow analysis and includes everything needed to perform *in vitro* digestibility tests; i.e. temperature controlled continuously mixed test vessels, optional vessels for carbon dioxide removal when methane analysis is performed, and a robust and reliable gas measuring system with a resolution of approximately 2 ml or 9 ml. In a study where three different ways of measuring the biochemical methane potential of cellulose were tested, the Gas Endeavour´s predecessor, AMPTS (Automatic Methane Potential Test System), provided the highest accuracy and repeatability (Esteves et al., 2011). Examples of studies where the AMPTS has been used are: investigation of methane potential from algae farming on available sludge streams from a waste water treatment plant (Rusten & Sahu, 2011), evaluation of different pre-treatments of sugarcane bagasse (Badshah et al., 2012) and evaluation of the effects from different chemical and biological additives on a substrate mixture (Strömberg et al., 2011).

In this study the system is presented, and the results of a long term *in vitro* ruminant fermentation are discussed and compared with the classical VOS procedure. These tests were performed with the predecessor of the new Gas Endeavour.

System

The Gas Endeavour has been used to perform several methane potential tests for biogas production on various types of substrates, as well as a number of *in vitro* digestibility studies with ruminant feeds. As can be seen in the below figures, the Gas Endeavour is available in various configurations. In Fig. 1 and 2, the unit on the left-hand side is the gas detection unit where measurements take place and data is stored. The unit in the middle is a CO_2 removal unit and consists of 15 small vessels with 3M NaOH. The unit on the right-hand side is the incubator with test vessels.

Figure 1 The Gas Endeavour with 250 ml bottles in a thermostatic shaking water bath.

Figure 2 The Gas Endeavour with 500 ml bottles and mechanical agitation in a thermostatic water bath.

The system is available with a 2- or 9-ml measurement resolution (Fig. 3 and 4), and has either 500-ml bottles with mechanical mixing and a regular thermostatic water bath, or 250-ml bottles in a shaking thermostatic water bath. The system can also be set up in a way to measure both total gas and methane simultaneously, by using two flow cells per bottle with a

CO_2 removal unit in between (Fig. 5). The detection unit, which can be seen on the left-hand side of Fig. 1 and 2, is a gas measurement unit where gas is collected in a flow cell by water displacement. When a pre-defined gas volume has been accumulated, the cell opens and releases the gas which is registered in the embedded CPU. Every opening corresponds to roughly 2 ml (or 9 ml, depending on the resolution) of gas and for each opening the ambient temperature and pressure are registered for calculations of normalised values (0°C, 1 atmosphere and zero moisture content).

Figure 3 Flow Cell Unit with 2 ml measurement resolution.

Figure 4 Flow Cell Unit with 9 ml measurement resolution.

Figure 5 The Gas Endeavour can be set up in a way to monitor both total gas and methane production simultaneously.

Among other applications, the Gas Endeavour can be used to conduct ruminant fermentation trials, feed additive studies, monogastric nutrition trials, biodegradability and compostability tests, greenhouse gas emission studies, silage studies, specific anammox activity tests, biochemical oxygen demand (BOD) analyses, aerobic and anaerobic respiration tests, and determining the dynamic profile of the target analysis.

A long-term *in vitro* feed digestibility test was performed with the predecessor of the Gas Endeavour, where the accumulated gas volume was correlated to the organic matter digestibility of six standard samples and a straw sample.

Materials and methods

The incubation intended to imitate the standard VOS 96-h procedure, with a set of six calibration samples that are normally included in each run. The incubation involved both gas measurement and gravimetric determination of organic matter disappearance in the incubation vessels. It was performed in conjunction to the lab's weekly routine for rumen *in vitro* organic matter digestibility (IVDOM) determinations of forage samples according to the 96-h VOS procedure (Lindgren 1979; Åkerlind et al., 2011). Proportions of rumen fluid, buffer and sample were similar to the VOS procedure with 10 ml rumen fluid, 290 ml VOS buffer ((Lindgren, 1979), containing per litre: 8.50 g $NaHCO_3$, 5.80 g K_2HPO_4, 0.50 g $(NH_4)_2HPO_4$, 1.00 g NaCl, 0.50 g $MgSO_4$•7 H_2O, 0.01 g $FeSO_4$•7 H_2O and 0.10 g $CaCl_2$) and 4 g of air-dry sample.

A set of six calibration samples with IVDOM values of 686 to 901 g/kg OM, that are included in each IVDOM batch at the lab, were incubated in duplicate and so was a barley straw sample with IVDOM value of 505 g/kg OM. A single blank bottle with 290 ml VOS buffer and 10 ml rumen fluid was also included.

The rumen fluid was from a maintenance fed non-lactating cow and collected in the morning. Handling of rumen fluid and buffer was similar to the lab's IVDOM procedures with straining of rumen fluid through a 1-mm screen, mixing with buffer and dispensing into incubation bottles without previous CO_2 flushing. Incubation was conducted over 96 hours.

After termination, each bottle was split into three glass filter tubes with porosity P1 (100-160 µm) and rinsed according to the VOS procedure with hot water and acetone. The samples were then dried overnight at 103°C and ashed for 3 h at 500°C according to the standard procedures for VOS to get a measure of remaining organic matter amount and hence organic matter digestibility in vitro.

Results and discussion

The *in vitro* digestibility test was performed at the Department of Animal Nutrition and Management, SLU, Uppsala, with the predecessor of the new Gas Endeavour. The accumulated gas volume was monitored over time, and remaining organic matter amount was measured after the incubation period. Table 1 shows average organic matter digestibilities, obtained with the VOS method for many replicates and for the actual batch (shown as mean from two water baths). Presented is also organic matter digestibility (OMD) obtained with the Gas Endeavour, together with the final gas amount. Average results of accumulated gas volume over time are presented in Figure 6 with standard deviation of duplicates.

Table 1 Vessel contents, organic matter digestibility in % (VOS) at lab as average of numerous replicates and as measured with actual VOS batch. OMD from the Gas Endeavour was determined gravimetrically, similar to the VOS procedure

Bottle	Sample	Sample (gram)	Strained rumen fluid (ml)	VOS buffer (ml)	VOS long term average	VOS in actual batch	OMD in Gas Endeavour	Final gas (ml)
1	Grass S1	4.00	10	290	73.4	72.8	75.0	379
2	Grass S1	4.00	10	290	73.4	72.8	75.2	379
3	Grass S2	4.00	10	290	78.8	77.2	79.6	380
4	Grass S2	4.00	10	290	78.8	77.2	79.5	409
5	Grass S3	4.00	10	290	68.6	68.3	70.3	309
6	Grass S3	4.00	10	290	68.6	68.3	70.9	336
7	Grass S4	4.00	10	290	81.2	80.6	82.0	291
8	Grass S4	4.00	10	290	81.2	80.6	80.2	275
9	Grass S5	4.00	10	290	84.0	80.7	84.2	388
10	Grass S5	4.00	10	290	84.0	80.7	82.9	352
11	Grass S6	4.00	10	290	90.1	88.0	88.2	491
12	Grass S6	4.00	10	290	90.1	88.0	89.5	470
13	Straw	4.00	10	290	-	50.5	57.1	171
14	Straw	4.00	10	290	-	50.5	57.4	172

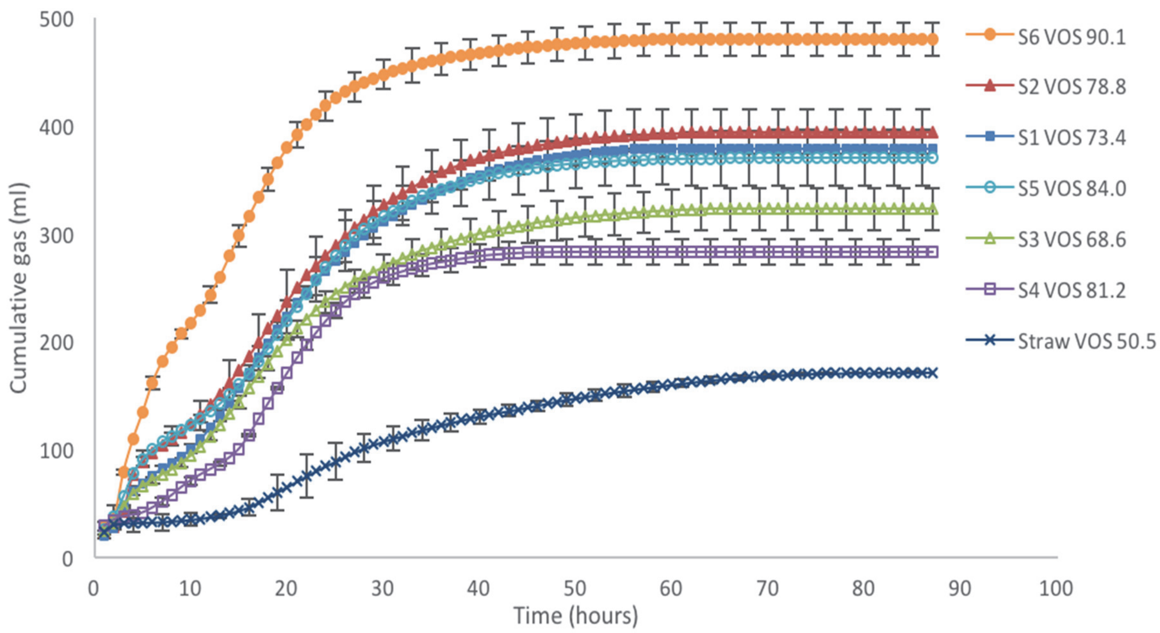

Figure 6 Accumulated gas volume over time, with final pH value.

There was a negligible amount of remaining organic matter in the blank (15 mg compared to 366-1565 mg in the samples) and the accumulated gas volume was 24 ml. The blank was not considered in this compilation. The Gas Endeavour resulted in a slightly higher remaining organic matter amount whereby the relative error compared to the VOS analysis was *circa* 3%. A clear difference in accumulated gas volume and gas production kinetics can be seen between the various grasses and straw. Figure 7 shows how the correlation between endpoint gravimetric OMD and gas volume changes for each hour. As can be seen with these samples, it would not pay off to measure gas for more than approx. 30 h. After that, the correlation decreases, which could be caused by microbial recycling and a more unpredictable gas production. Overall, however, the results from the two methods were well correlated, with a correlation of approximately 0,88 for a standard *in vitro* rumen fermentation of 24 hours.

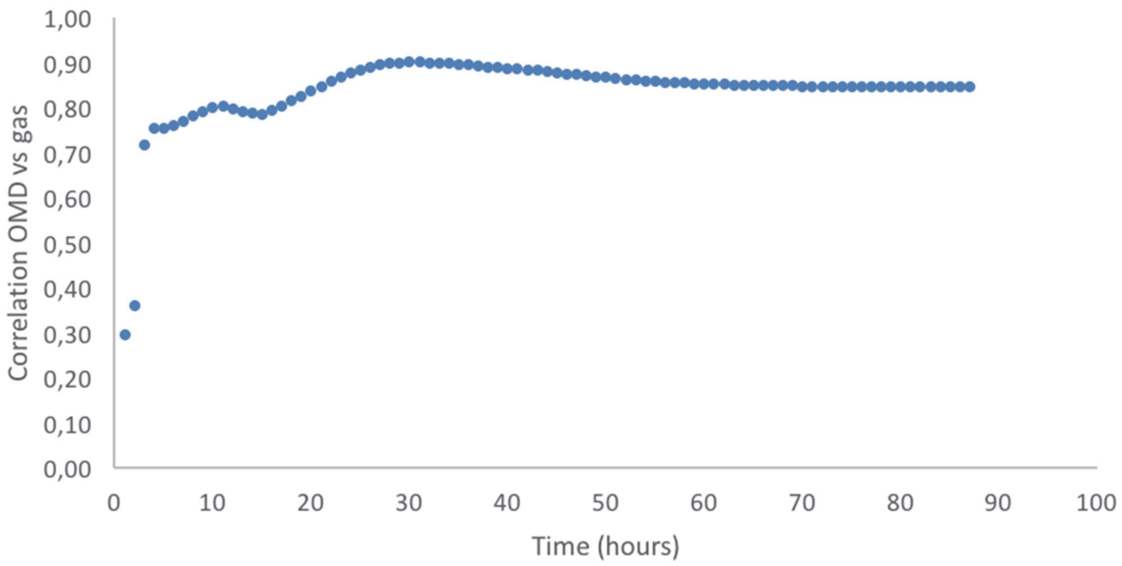

Figure 7 Correlation between cumulative gas volume per hour and endpoint organic matter digestibility.

Conclusions

In this work, the new Gas Endeavour of Bioprocess Control was presented. Result of a long term *in vitro* feed digestibility test, performed with the predecessor of the Gas Endeavour, are presented. A clear correlation between gas production and OMD was found, with decreasing correlation after 30 hours of incubation.

The newly developed Gas Endeavour offers the additional possibilities to study gas composition in real-time. It has a more accurate measurement of low flow of highly water soluble gases, while maintaining the proved measurement principle of its predecessor.

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