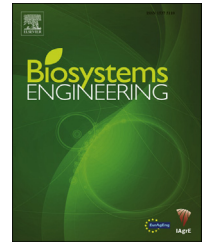


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Research Paper

Design and test of an artificial reference cow to simulate methane release through exhalation



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To mitigate methane emission from dairy cows, a technique is needed to evaluate individual methane emission from a large number of cows under practical conditions in barns. For developing such a measurement technique, a known reference source that can simulate cow exhalation of methane would be a powerful tool to improve and validate measurement methods. The objective of this research was to design, construct, and test an artificial reference cow (ARC). We built a device that simulated exhaling and inhaling cycles and eructation. The ARC consisted of a cylinder in which methane was injected by mass flow controllers and ejected by a piston in the cylinder. The methane mass balance of the ARC, defined as the difference between the mass controllers imposed input and measured output, was tested under three settings. Methane concentration release patterns produced by five simulated cows were compared to patterns measured from real cows. Average methane concentration in exhaled gas had a mean difference of 2.8% between measured and predicted results. The output methane mass was strongly linearly related to the input methane mass. Methane concentration release patterns produced by the five simulated cows had a sinusoidal curve with similar time interval and comparable methane concentration level as real cows. It is concluded that the ARC properly represented the methane production release, and that the system precisely controlled methane concentration and production. The ARC can be used as a known reference source to develop practical methane measurement methods.

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1. Introduction

Efforts to mitigate methane emission from dairy cows are critical to reduce the dairy industry's contribution to the production of greenhouse gases and subsequently to global warming. Among ruminants, dairy cows have been identified as the major producer of methane emission as they account

for 15% of the global methane emission budget (Gerber, Vellinga, Opio, Henderson, Steinfeld, 2010; Lassey, 2007; Lassey, Ulyatt, Martin, Walker, & Shelton, 1997). The release of methane by dairy cows also represents a loss of energy for the animal. Mitigating methane emission from dairy cows will therefore benefit not only the environment but may also improve the feed efficiency of cows.

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Nomenclature

BF	breath frequency [times per minute]
C_a	methane concentration in ambient air [ppm]
C_e	mean methane concentration in the exhaled gas [ppm]
$C_{ex,in}$	methane concentration inside the cylinder of ARC during exhaling or inhaling [$g\ l^{-1}$]
F	methane flow rate controlled by MFC [$l\ min^{-1}$]
M	methane mass injection rate by MFC [$g\ s^{-1}$]
M_{in}	ARC's methane input and output [g]
M_{out}	ARC's methane input and output [g]
T_e	measurement time [min]
T_v	tidal volume [l]
V	gas exchange volume rate [$l\ s^{-1}$]
x	methane mass accumulation rate in the cylinder [$g\ s^{-1}$]
ρ	methane density [$g\ l^{-1}$]

The heritability of methane emission in ruminants was demonstrated in a large scale study with sheep based on respiration chamber measurements (Pinares-Patiño et al., 2013). There are strong indications that methane emission from dairy cows can be mitigated by breeding cows with low methane emission but a breeding approach requires a large number of methane production records from individual cows (De Haas et al., 2011). The high number of cow datasets required for breeding implies that farm measurement methods are needed. In research, the respiration chamber method is considered as the gold standard for determining individual methane production from cows. However, this laboratory method is not suitable for measurements on a large number of animals. Currently, three main techniques are used to measure methane emission from individual cows under farm conditions: 1) the sulphur hexafluoride (SF_6) tracer method (Grainger et al., 2007; Johnson, Huyler, Westberg, Lamb, & Zimmerman, 1994; Lassey, Pinares-Patiño, Martin, Molano, & McMillan, 2011), 2) the breath concentration method (Garnsworthy, Craigon, Hernandez-Medrano, & Saunders, 2012; Lassen, Løvendahl, & Madsen, 2012), and 3) the GreenFeed method (Greenfeed system, 2014). The SF_6 method is an invasive method that requires inserting a permeation tube into the rumen and attaching a collection canister around its neck, and as such not suited for large scale farm application. In contrast, the breath concentration method is a non-invasive on-farm method for individual cows, based on monitoring cows' exhaled air in concentrate feeders when visiting the milking parlour. This method monitors the methane concentration in the mixed air near a cow's head during a short period of feeding, and can only be considered as an indirect measure of a cow's actual daily methane production. Conversely, the GreenFeed method is monitoring both methane and carbon dioxide fluxes emitted from the dairy cows when visiting a feeder. However, compared to the average 10 h ruminating per day per cow, monitoring intervals based on the feeding or milking period are relatively short to determine the daily methane production. Each of the three mentioned methods has limitations either related to its technical complexity and

costs or to the short sampling periods. Further improvement of these methods and new concepts of cow monitoring are helpful in implementing a breeding strategy.

The measurement accuracy of the SF_6 tracer method, the breath methane-measurement method, and the GreenFeed method is generally validated by comparing the recorded cow values to those derived for the same cows from the respiration chamber method. This method is set as the reference for developing new methods because of its accurate measurement results (Aulick, Arnhold, Hander, & Mason, 1983; Blaxter, Brockway, & Boyne et al., 1972; Hellwing, Lund, Weisbjerg, Brask, & Hvelplund, 2012; Pinares-Patiño and Waghorn, 2012; Rosenbaum, Makoff, & Mills, 1969). In this method a cow is housed in a small confined chamber for several days to monitor its methane release. However, a small and restricted chamber can modify a cow's behaviour, reduce their feed intake and consequently influence methane production (Garnsworthy et al., 2012; Lassen et al., 2012). Moreover, use of the respiration chamber is limited by the time it takes to train an animal for the specific measurement conditions, the number of animals it can handle, and the large expense of building and maintaining a chamber. In short, a respiration chamber is a time-consuming and costly reference method to manage, and may produce biased results when compared to methane release under farm conditions. Considering these shortcomings, a more practical reference technique would be helpful in research efforts aimed at developing improved farm methods for monitoring cow methane emission. Such an alternative technique could be provided by developing an artificial reference cow (ARC) that mimics the methane release of real cows with known pre-set quantities and that can be used to evaluate methods under a wide variety of practical conditions in barns. This would enable instant evaluation of new monitoring methods under barn conditions with high precision, contrary to using real test cows whose methane flux has to be determined in respiration chambers before or afterwards the barn test.

The objective of this study is to design, construct, and evaluate an ARC that meets the following requirements:

- The ARC should be capable of providing cow exhalations with pre-set methane production rates at a high precision level;
- The ARC with known methane production rates and should be capable of mimicking methane release patterns, exhalation volumes and temperatures that are typical for real cows;
- The ARC should operate accurately and stably for a long period under a wide variety of practical laboratory and barn conditions.

The first section of this paper (Section 2.1) describes the schematic and working principle of the ARC we developed, and explains how the basic properties of the ARC were designed to mimic real cow exhalation properties. The following sections (Sections 2.2 and 2.3) describe the equipment and equations used in the experimental evaluation of the system. The setup of the ARC evaluation was based on comparing controlled input and measured output at different parameters settings, and comparing realised methane

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