Chapter 6
Methane Production in Ruminant Animals

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Abstract Agriculture is a significant source of GHGs globally and ruminant livestock animals are one of the largest contributors to these emissions, responsible for an estimated 14% of GHGs (CH_4 and N_2O combined) worldwide. A large portion of GHG fluxes from agricultural activities is related to CH_4 emissions from ruminants.

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Both direct and indirect methods are available. Direct methods include enclosure techniques, artificial (e.g. SF_6) or natural (e.g. CO_2) tracer techniques, and micrometeorological methods using open-path lasers. Under the indirect methods, emission mechanisms are understood, where the CH_4 emission potential is estimated based on the substrate characteristics and the digestibility (i.e. from volatile fatty acids). These approximate methods are useful if no direct measurement is possible. The different systems used to quantify these emission potentials are presented in this chapter. Also, CH_4 from animal waste (slurry, urine, dung) is an important source: methods pertaining to measuring GHG potential from these sources are included.

Keywords GHGs · Animals · Direct and indirect emission · SF_6 · CH_4

6.1 Introduction

Agriculture contributes more than 25% to the total greenhouse gases (GHGs) globally, and ruminant livestock animals are one of the largest contributors to these emissions, responsible for an estimated 14% of GHGs worldwide (Tubiello et al. 2014). In non-industrialised countries, emissions from livestock (methane and nitrous oxide

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combined) may be the most important source of GHGs. However, measurement–though critical for meeting international obligations and for assessing mitigation options–is not commonly undertaken in most countries.

Many methods are available for estimating enteric methane (CH_4) production in ruminant animals. They can be broadly classified into two groups–direct and indirect. The important distinction is that direct methods measure CH_4 produced by the ruminant animals in some manner, whereas indirect methods infer CH_4 production from parameters that are associated with CH_4 production in the ruminant animals. In all cases, the methods have strengths and weaknesses and need to be selected with care for the particular objective in mind. The choice of method will depend on available financial and technical resources and the purpose of the measurement, including whether interactions between ruminant animals and environment are important to the research question. If it is impractical to use any of the direct methods, then less precise indirect methods can be used. This chapter will include examples of both direct and indirect measurement methods. Direct methods include enclosure techniques, artificial (e.g. SF_6) or tracer techniques based on herd-level experiments in natural ventilated cattle housings (e.g. using CO_2 as internal tracer), and micro-meteorological methods using open-path lasers. Under the indirect methods, emission mechanisms are understood, where the CH_4 emission potential is estimated based on the substrate characteristics and the digestibility (i.e. from volatile fatty acids). These approximate

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methods are useful if no direct measurement is possible. This chapter provides an overview of the different systems used to quantify these emission potentials are presented. Also animal wastes are an important source of CH_4 from animal waste (slurry, urine, dung) is an important source; thus, methods pertaining to measuring GHG potential from these sources are also included.

6.2 Direct Measurements

6.2.1 Enclosure Techniques

All enclosure methods rely on the principle of measuring either continuously or intermittently, the concentration of CH_4 in and the total flow of air from around the animal. Methods vary in technical complexity, ease of operation, and precision.

6.2.1.1 Total Enclosure of Animal

The technique of open-circuit indirect respiration calorimetry has been routinely used with many species of ruminant animals to determine partition of dietary energy. This involves the measurement of oxygen (O_2) consumption and carbon dioxide (CO_2) production. For ruminants, the emphasis is quite different–determination of total CH_4 production, arising largely from rumen fermentation to provide more precise estimates of relationships between dietary energy intake and CH_4 production.

Models to estimate national and global CH_4 emission from sheep and cattle at the farm level are mostly based on data of indirect calorimetric measurements, which most precisely measure the relationship between enteric CH_4 and feed intake (Johnson and Ward 1996). Respiration chambers are used to measure CH_4 at an individual animal level–their use is technically demanding, and the number of animal measurements possible will be determined by the availability of physical infrastructure (number of chambers). However, these systems provide the most precise measurements on enteric CH_4 production.

Although design of the chambers varies, the basic principle remains the same. Chambers are constructed to house the subject animals, which are then sealed off and their environment is controlled (Plate 6.1). All open-circuit chambers are characterised by an inlet and exhaust, so animals breathe in a one-way stream of air passing

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through the chamber space. Air can be pulled through each chamber by exhaust fans, generating a negative pressure within the chamber, which minimises loss of air from the chamber. However, in practice, CH_4 can still be lost from chambers (down the concentration gradient) that are imperfectly sealed, so, Gas recovery is not a maintenance task. Thresholds for chamber temperature (<27 °C), relative humidity (<90%), CO_2 concentration (<0.5%) and ventilation rate (250–260 l min^{-1}) have been described (Pinares-Patiño et al. 2011). Although these parameters may be varied, it is very important to ensure that subject animals remain in their thermo-neutral zone while being measured, or voluntary intake is likely to be compromised. In practice, chambers in tropical and sub-tropical regions will need to be fitted with air conditioning units, which will also provide a degree of dehumidification and a ventilation system. This ensures that chambers can be maintained at a constant temperature or at near ambient temperatures so as to capture the normal diurnal pattern of CH_4 production (Tomkins et al. 2011). Feed bins and automatic water systems may also be fitted with electronic scales and meters, respectively, to monitor feed and water intake during CH_4 measurement periods.

CO_2 and CH_4 concentrations are measured by sampling incoming and outgoing air, using infrared laser gas analysers, infrared photoacoustic monitors or gas chromatography systems. The other essential measurement is airflow over the measurement period, which is generally either a 24 or 48 h period. The accuracy and thus the validity of measurements are dependent on the sensitivity of the gas analysers used and routine calibration of these devices. Direct calibration of chambers is performed by releasing a known volume and concentration of standard gas to estimate recovery values (Klein and Wright 2006). Alternatively, a gas is released at a known rate until the concentration inside the chamber has equilibrated. Measurements are also influenced by the environmental conditions including temperature, humidity, atmospheric pressure and incoming air composition, and these parameters need to be measured and recorded as part of the measurement process. The calibration of the gas analysers must be accurate and reproducible for long-term usage.

Apart from technical complexity, an important limitation of the technique is that normal animal behaviour and movement is restricted when animals are kept in the respiration chambers (Table 6.1). Animals to be measured will benefit from acclimation in chambers prior to confinement and measurement to minimise alterations in their behaviour: such as decreased feed intake. Even so, there is clear evidence that this will happen in a small proportion of animals regardless of training, which should be borne in mind when interpreting data (Robinson et al. 2014). Using transparent construction material in chamber design allows animals to have visual contact with the other housed animals. The chamber should be sufficiently rigid to tolerate normal animal behaviour and if possible, a metabolism stall should be provided within the chamber to restrain the animal. Provisions should of course be made for feeding and watering the animal.

Substantial costs will be incurred with construction and maintenance of the open-circuit respiration chambers; the requirement for high-performance and sensitive gas analysers and flow meters need consideration in design and construction. Only a few
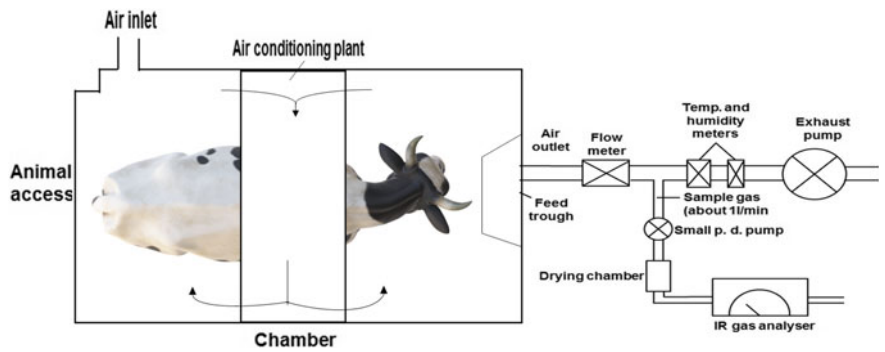
Plate 6.1 Schematic diagram of open-circuit calorimeter to measure CH_4 production in animals

Table 6.1 Recommended dimensions (m) of chambers for different livestock classes

	Height (m)	Width (m)	Length (m)	Approx. Volume (m^3)
Dairy cows	2.0	2.0	3.6	14.4
Growing beef cattle	1.8	1.8	3.0	9.7
Sheep and goats	1.6	1.0	2.0	3.2

animals can be used for measurements within chambers at any one time. Nevertheless, respiration chambers are well suited to study the differences between treatments in response to mitigation strategies and continue to be regarded as the "gold standard" for measuring individual animal emissions.

Air conditioners comprising of a cooling coil and associated refrigerator are essential in hot regions; air filter and coil heater are optional.

- Air ducting.
- Metabolism stalls with suitable feeding and watering facilities.
- Vacuum pump with suitable valve system to adjust air removal rate.

Measurement of Air Volume

Several methods are available to determine the volume of air passing through the chamber. The simplest device (which requires no electrical supply) is a commercial dry gas meter, which gives a direct measure of total gas flow. Other alternatives include electronic turbine flowmeters and venturi apparatus. These give instantaneous rates of gas flow and are highly reliable. However, the need to integrate the individual airflow rates and the relative sophistication of such apparatus precludes their use in laboratories without adequate and reliable electrical supply. It is necessary to correct the total flow of gas to Standard Temperature and Pressure (STP). This requires a determination of atmospheric pressure and temperature.

Measurement of CH$_4$ from Chamber Exhaust

The exhaust air needs to be analysed for CH$_4$ concentration. Several possibilities exist for CH$_4$ measurement. Most commonly, gas samples will be taken at regular (4–12 min) intervals from each chamber in turn, for ~2 min. (to allow for adequate flushing of the residual from the previous sample) using an automated switching device. The actual measurement time required will depend on the measurement apparatus itself (as outlined in the previous section). A possible alternative and simpler method is to acquire a small, representative subsample of the chamber gas over the entire run using a low flow rate pump (such as a peristaltic pump) and stored in gas-impervious (e.g. polyvinyl fluoride (PVF); Tedlar®) bags. It is important that the flow rate of the aliquot collection is constant, thereby providing an integrated representation of the entire collection. After the run has been completed, CH$_4$ concentration may be measured by the methods outlined in Sect. 6.8.

6.2.1.2 Head Box

Without the use of a tracer that moves with CH$_4$, it is essential to collect all the expired and eructated (belching) gas. Drawing a stream of air past the muzzle of the animal by enclosing the animal's head in a hood can do this. The method requires considerable training of the animals. The hood can be made sufficiently large so that it is much less restrictive–thus more easily accepted. Additionally, the animal can be fed and have access to water during the collection of gas samples. In both methods, the principle concern is to have a sufficient outflow of gas to ensure there is lower gas pressure in the hood and gas lines and that all the "leaks" are inward. However, there is still considerable scope for part of the sample to leak, up the concentration gradient and be "lost". Hence, performance of gas recoveries is essential in properly calibrating the method. With the accuracy of available analytical equipment, the dilution of CH$_4$ by air drawn past the animal's head does not present a problem.

The ventilated hood system is a simplification of a whole animal respiration chamber, as it measures the gas exchange only from the head rather than the whole body. Modern ventilated hood systems, applied for CH$_4$ measurements, have been used in Japan, Thailand (Suzuki et al. 2007, 2008), the USA (Place et al. 2011), Canada (Odongo et al. 2007), and Australia (Takahashi et al. 1999). Recently, Fernández et al. (2012) described a mobile open-circuit respiration system. The ventilated hood system used by Suzuki et al. (2007, 2008) consists of a head cage, the digestion trial pen, gas sampling and analysis, behaviour monitoring, and data acquisition system. Like the whole animal chambers, it is equipped with a digestion pen for feed intake and excreta output measurements. An airtight head cage is located in front of the digestion pen and is provided with a loose-fitting sleeve to position the animals' head. Head boxes are provided with blowers, to move the main air stream from the inlet to the exhaust. Flow meters correct the air volume for temperature, pressure and humidity. Air filters are set up to remove moisture and particles out of the gas samples, which are sent to the gas analysers (Suzuki et al. 2007). The mobile

system of Fernández et al. (2012) contains a mask or a head hood connected to an open-circuit respiration system, which is placed on a mobile cart.

The ventilated hood system is a suitable method under some circumstances: especially where open-circuit chambers are not viable. A critical limitation of the hood system is that extensive training to allow the test animals to become accustomed to the hood apparatus is absolutely essential–thus, while it can be used to assess potential of feeds, it is not suitable for screening large numbers of animals, primarily because of the high degree of training required. A further consideration is that hoods capture only measurements of enteric methanogenesis, excluding the proportion in flatus.

Design of Hoods

The hood should be designed to provide sufficient feeding space and enough room for the animal to move its head in an unrestricted way. A wide variety of materials may be used to build a box that is reasonably airtight. The most common materials used are wood and metal. While they can be custom-built, it is also possible to use plastic or metal drums or pre-constructed packing crates. It is a major advantage to have a clear removable panel to provide access for feeding and for checking the animal. This clear panel helps to maintain normal animal behaviour, particularly if other animals are visible to the experimental animal during the period it is in the hood. The animal should be restrained while its head is in the hood and the design of the hood depends on the facility available for restraint. For example, if animals are held in metabolism cages where they cannot turn around, a canvas sleeve can be fitted around the neck and connected to the hood as shown in Plate 6.2. This allows the animal to stand, eat and lie down during the measurement period. It may also be necessary to restrain the animal within the hood by means of a halter or collar. Hoods may also be built around yokes or even head bales at the end of a working race. It is desirable to minimise the amount of air leakage around the neck and head: a sleeve is an effective means of achieving this. This can be tied around the neck using a drawstring. The sleeve can be constructed from any material, but canvas or heavy cotton is most suitable. The length of the sleeve should be enough to allow the animal to stand up, lie down and have unrestricted access to feed and water.

An example for sheep (arrangement similar to that shown in Plate 6.2) uses a box made of 9 mm plywood that has solid sides 0.9 m × 0.4 m top and bottom (0.4 m × 0.6 m). The front and back panels (0.9 m × 0.6 m) have windows 0.25 m × 0.25 m for the animal's head and 0.5 m × 0.5 m for feeding and observation. A removable clear plastic or Perspex panel 0.6 m × 0.6 m is required for the feeding/observation window. The dimensions of the box can be varied to accommodate standard feeders, a water trough and the layout of the animal housing. The length of the sleeve should be around 0.35 m and will taper from a diameter of 0.25 m at the point where it is attached to the hood to 0.15 m so as to fit over the animal's head and be secured around the neck. The dimensions of the hood should be increased for cattle (approximately 3 times larger than for sheep). This will vary considerably according to the size
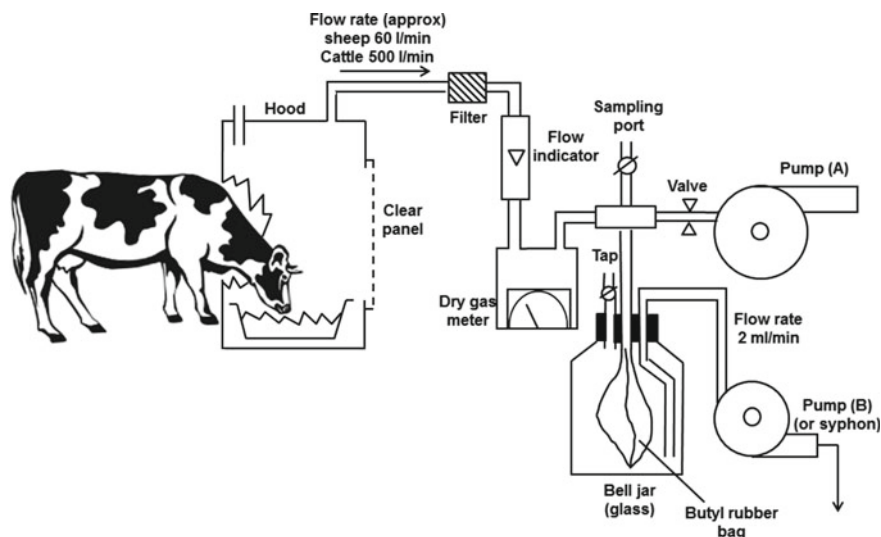
Plate 6.2   Gas sampling from hood: subsampling over 24 h for subsequent analysis

of the cattle and the type of feed (diet). The basic principle for the hood is that it should be sufficiently large for the animal's comfort while maintaining the ability to feed concentrate and roughage diets. Attention should also be given to minimise the places where gas can leak from the system

Gas sampling: The methods for collecting the gas sample, including gas lines, pumps and meters, are similar irrespective of whether hoods or chambers are used.

Sampling and airflow: Airflow of $50–70 \, \mathrm{l \, min^{-1}}$ is suggested for measuring CH_4 production in sheep. This gives concentrations of between 100 and 500 ppm (v/v) of methane in the airflow from the hood, but this may need to be modified, depending on the range over which the measuring device exhibits a linear response. For example, for cattle a flow rate of anywhere from 9 to 21 $\mathrm{l \, sec^{-1}}$ the actual flow rate will be determined by the concentration range deemed most suitable for the gas measurement apparatus used, which may be indicated, depending on the measurement device, live weight of the animal and level of feeding. These levels of gas flow will also be sufficient to provide the animal with fresh air and maintain CO_2 levels below 1%. Gas flow rates are easily controlled by altering the speed of the pump or even by an in-line flow restrictor but must not be altered during a run. A single pump can be used to sample a number of animals providing each sampling line has independent control of flow rate and a separate airflow meter. A schematic diagram is shown in Plate 6.2.

Sampling lines: The gas sampling lines can be secured overhead. The diameter of the tubing should be between 1.5 and 2.0 mm ID for sheep and 2–5 mm ID for cattle and can be constructed of materials such as copper, PVC or flexible rubber or plastic hoses, although CH_4 will leak from plastic or PVC tubing, and the use of these materials in a sampling line is better to avoid if possible.
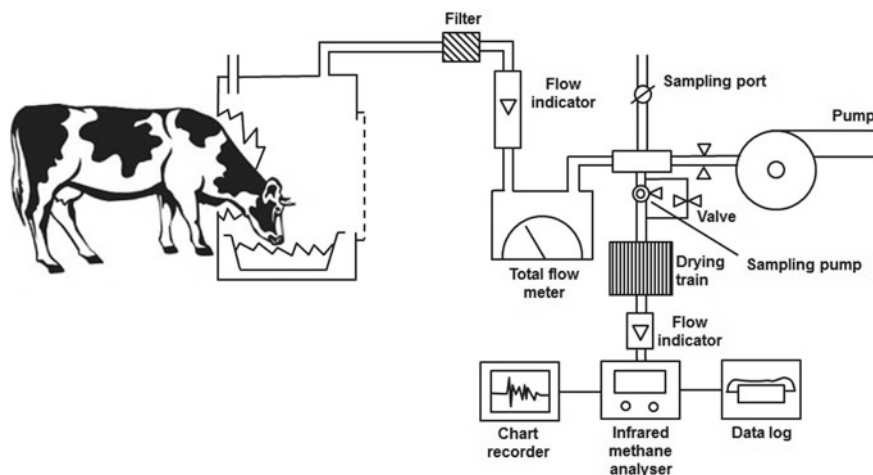
Plate 6.3 Gas sampling and continuous analysis of a subsampled air stream

Filter: It is advisable to have a simple filter system in the main sampling line in order to remove insects and feed particles that may enter the line. A jar containing plastic scourers or glass wool is adequate.

Gas flow measurement: The total volume of gas drawn past the animal must be measured accurately using a commercial dry gas meter.

Analysis and Subsampling Systems

Continuous gas analysis: It is possible to analyse the CH_4 content of the gas from the animal by incorporating an infrared gas analyser in the main line, or in a stream of gas taken from the main line (see Plate 6.3). With this system, the output from the meter needs to be recorded continuously and integrated over time.

Calibration of this equipment requires relatively large quantities of gas, and this can present a potential source of error and an on-going cost. In addition, it requires relatively sophisticated recording and integration equipment or a data logger. Placing the infrared gas analyser in the main line is not recommended unless there is a specific need to measure changes over time.

Time scale for measuring CH_4 production: The extrapolation of short-term measurements of CH_4 production by subsampling over only part of the day can be very misleading. There is considerable variation in the rate of CH_4 production during the day, and it can change considerably after feeding. Unless the measurement is for specific screening purposes, and the time of sampling is standardised with respect to feeding and other animal husbandry practices, measuring for periods of less than 24 h is not recommended.

Calculation: The production of CH_4 is calculated by multiplying the concentration of CH_4 in the subsample by the total volume of gas passing through the hood. For example, a daily airflow of 86,000 l past the head of a sheep with a CH_4 concentration of 200 ppm (v/v) gives a daily production of 17.2 l day^{-1} (0.77 mol day^{-1}). In cattle, corresponding values might be a daily airflow past the animal of 720 000 l and a concentration of 250 ppm (v/v). In this instance, CH_4 production would be 180 l day^{-1} (8.04 mol day^{-1}).

Greenfeed[®] Emission Monitoring Apparatus

Greenfeed[®] is a patented device (Zimmerman and Zimmerman, 2012) that measures and records short-term (3–6 min duration) CH_4 emissions from individual cattle repeatedly over 24 h by attracting animals to the unit using a "bait" of pelleted concentrate. By being available 24 h/day, potential sampling bias is reduced, and the technique has been shown to provide comparable estimates to that produced by both respiratory chamber and SF_6 techniques (Hammond et al. 2013). However, a significant limitation of the technique is the requirement to supply an "attractant" to lure the animal to use the facility, consisting of up to 1 kg of concentrate pellets per day. This will certainly affect DMP and may also alter VFA profiles or the overall digestibility of the diet consumed. Attempts to use energy-neutral attractants, such as water, have proven equivocal (J Velazco pers. comm.).

6.2.1.3 Polytunnel

The polytunnel is an alternative to respiration chambers although operation and measurements are somewhat simpler. Methane emissions from individuals or small groups of animals can be acquired under some degree of grazing. This allows test animals to express normal grazing behaviour, including diet selection over the forages within the polytunnel space. It has been used in the UK to measure CH_4 emissions from ruminants under semi-normal grazing conditions. Murray et al. (2001) report CH_4 emissions from sheep grazing of two ryegrass pastures and a clover/perennial ryegrass mix pasture using this methodology. Essentially, polytunnels consist of one large inflatable or tent-type tunnel made of heavy-duty polyethylene fitted with end walls and large diameter ports. Air is drawn through the internal space up to 1.0 m^{3}s^{-1} (Lockyer and Jarvis 1995). In general, polytunnels may be used where emissions from fresh forages are of interest because animals can be allowed to graze a confined area of known quality and quantity. When the amount of available forages is depleted, the tunnel is moved to a new patch.

Airflow rate can be measured at the same interval as the CH_4 levels are assessed or can be continuously sampled at the exhaust port (Lockyer 1997). Micropumps may be used to pass the exhausted air to a dedicated gas analyser or a GC (Murray et al. 2001). Data from all sensors can be sent to a data logger, which records flow rate, humidity and temperature within the tunnel, as well as gas production from

the livestock. Samples of the incoming and exhaust air can be taken as frequently as necessary, depending on the required accuracy. The samples can be either taken manually or by an automatic sampling and injection system.

The polytunnel system requires frequent calibration to assure a good recovery rate, which is performed by the same principle as the chamber technique. Methane measurements can be collected over extended periods of time. Fluctuations can be expected to occur due to changes in animal behaviour, position relative to the exhaust port, internal temperature and relative humidity and grazing pattern of the animal: eating, ruminating or resting (Lockyer and Jarvis 1995; Lockyer and Champion 2001). The polytunnel is suitable for measuring CH_4 emissions under semi-normal grazing conditions. It has been reported that the tunnel method gives lower readings of CH_4 concentration (15%), compared to the respiration chamber method, suggesting that animals actually consume less–this requires further investigation. Recovery rate is high in both systems, 95.5–97.9% versus 89.2–96.7% for tunnel and chambers, respectively (Murray et al. 1999). With an automated system, measurements can be performed with high repeatability. The polytunnel system is portable and can be used on a number of available pastures or to also browse shrubs. Its utility is limited by the inability to capture and record feed intake.

6.2.1.4   Portable Accumulation Chambers (PAC)

PACs consist of a clear polycarbonate box of approximately 0.8 m^3, open at the bottom and sealed by achieving close contact with flexible rubber matting. Methane production is measured by the increase in concentration, which occurs over approximately a 1 h period. PACs were designed to screen large numbers of sheep to identify potentially low and high methane-emitting individuals and thus develop estimates of the genetic parameter in sheep populations. This technique initially showed good agreement with respiratory chamber measurements (Goopy et al. 2009, 2011) and subsequent investigations demonstrated such measurements to be moderately repeatable in the field and to have potential for genetic screening of the animals (Goopy et al. 2014). Longer term comparisons of PAC measurements and respiratory chamber data, however, suggest that these two methods may be measuring quite different traits and further investigations are required before committing significant resources to pursue measurements using PACs (Robinson et al. 2015a, b).

6.3   Tracer Techniques

6.3.1   Use of SF$_6$ Bolus

The sulphur hexafluoride (SF_6) technique is a direct measurement of the CH_4 emission of individual animals. This technique can be performed on an individual animal

under normal grazing conditions and can also be employed under more controlled conditions where intake is measured and/or regulated.

The SF_6 principle relies on the insertion of a permeation tube with a predetermined release ratio of SF_6 into the rumen, via the mouth (per os). Air from around the animal's muzzle and mouth is drawn continuously into an evacuated canister connected to a halter fitted with capillary tube around the neck. Johnson and Ward (1996) provide a detailed description of the methodology.

The regulation of the duration of collection of each sample is achieved by altering the length and/or diameter of the capillary tube (Johnson and Ward 1996). Several modifications have since been reported with specific applications (Goopy and Hegarty (2004); Grainger et al. 2010; Ramirez-Restrepo et al. 2010). Most recently, Deighton et al. (2014) have described the use of an orifice plate flow restrictor which considerably reduces the error associated with sample collection and thus should be considered in preference to the traditional capillary tube flow restrictors. At completion of sample collection, the canisters are pressurised with N_2 prior to compositional analysis by gas chromatography. Enteric CH_4 production is estimated by multiplying the CH_4/SF_6 ratio by the known permeation tube release rate corrected for actual duration of sample collection and background CH_4 concentrations (Williams et al. 2011), which are determined by sampling upwind ambient air concentrations. Williams et al. (2011) emphasised the importance of a correct measurement and the reporting of the background concentrations, especially when the method is applied indoors. Methane is lighter ($16\,g\,mol^{-1}$) compared to SF_6 ($146\,g\,mol^{-1}$); therefore, it will disperse and accumulate differently depending on ventilation, location of the animals and other building characteristics.

This method enables sampling of gas concentrations in exhaled air of individual animals, and it also takes into account the dilution factor related to air or head movement. A significant limitation of this method, however, is the high within- and between-animal variation. Grainger et al. (2007) reported a variation within animals between days of 6.1% and a variation among animals of 19.7%. Pinares-Patiño et al. (2011), monitoring sheep in respiration chambers simultaneously with the SF_6 technique, reported a higher within ($\times 2.5$) and between ($\times 2.9$) animal variance compared to the chamber technique combined with a lower recovery rate (0.8 ± 0.15 vs. 0.9 ± 0.10). These sources of variation need to be taken into account in determining the number of repeated measures needed to obtain accurate results. Moate et al. (2015) have recently described the use of Michaelis–Menten kinetics to better predict the discharge rate of the SF_6 capsules, which should potentially reduce the error associated with estimating discharge rates and also prolong the useful life of experimental subjects through the improved predictability of discharge rates over much longer intervals.

The SF_6 technique gives animals the ability to move and graze normally on test pastures. This makes the method suitable for examining grazing management effects on CH_4 emission (Pinares-Patiño et al. 2007), but it does so at a cost. The SF_6 method is less precise, less physically robust (high equipment failures) and more labour-intensive than respiration chamber method (Plate 6.4).

Plate 6.4 Structure of the slow permeation bolus for releasing control amounts of SF_6
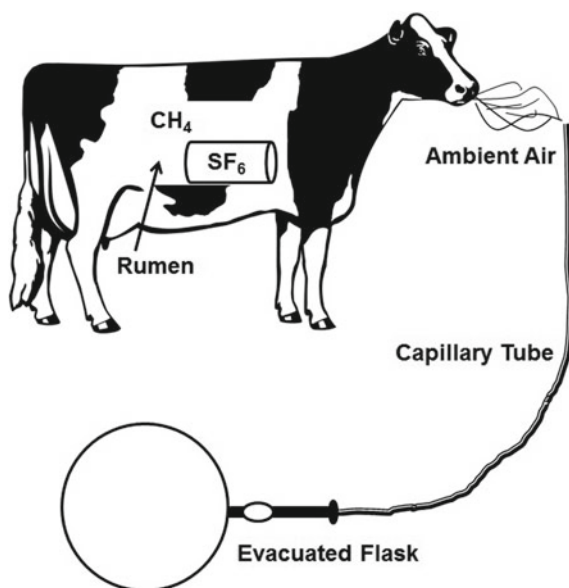
The permeation tubes consist of a closed stainless-steel tube capped at one end with a Teflon disc held in place with a standard Swagelok^{Tm} nut. The tube is filled with SF_6 while being cooled with liquid N_2 causing it to reach solid state on contact. It is then allowed to equilibrate to a fixed temperature in a water bath. Permeation rates are determined gravimetrically through repeated weighing on a precision balance over a period of several weeks. Typical permeation rates are of the order of 0.5–2.5 mg day^{-1}, although they may be considerably higher if circumstances dictate. Permeation tubes should be inserted into the rumen a minimum of 3 days prior to the first scheduled collection to allow steady-state conditions to be reached. Insertion can be accomplished with a balling/drenching gun or other similar methods for inserting boluses into the rumen.

A leather pad attached to the noseband of a halter serves as an anchor point for the sample line near the animal's nose and mouth. A small piece of plastic tubing is attached to a filter and oriented such that it is placed over one of the nostrils (see Plate 6.5). A filter (10 μm) connected to the upstream end of the sample line protects the flow restrictor from becoming plugged. Fastening the tube to the sides of the halter helps to protect the capillary tubing and reduces animal irritation. The capillary tube-collection flask connection should be via a quick-connect fitting to simplify flask exchange. A soft rope fastened around the neck with a clasp that can be attached to the collection flask helps stabilise the flask and takes pressure off the capillary tube.

The collection vessel should be large enough to accommodate the size of the desired sample (i.e. ~5 l for cattle), should be able to withstand a vacuum and should have a valve for sealing the flask. Immediately prior to use, all air should be removed
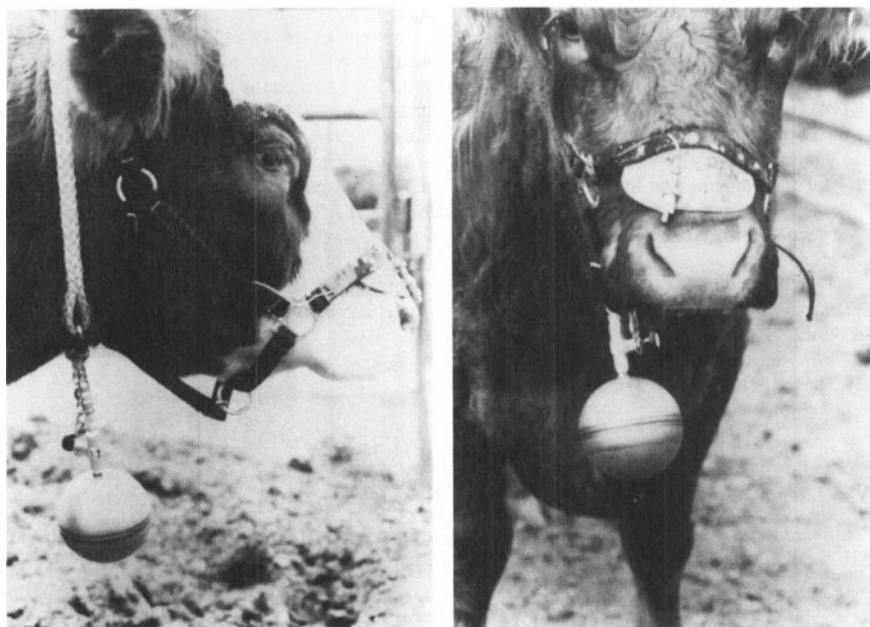
Plate 6.5   Position of the evacuated vessels on the animal

from the flask and the valve closed. After fastening the flask to the supporting neck rope and attaching the capillary tube, the time of day should be noted, and the valve opened. When the sampling time is complete, the flask is removed for analysis. If repeated collections are desired, another flask should be added after the first one is filled. It is recommended that many measurements are made on each animal, and that total 24 h emissions are also reported. The diameter and length of the capillary tubes needed depend on the rate at which sampling is desired. The size of the capillary tube bore should be such that the evacuated sample flask fills to about 1/2 atmospheric pressure over the desired sampling time. The flask pressure should be measured, and the flask then filled with N_2 to bring it to positive pressure (around 1.5 atmospheres). Both pressures need to be recorded to enable the extent of dilution by N_2 to be calculated.

The vessel samples can be quickly and accurately analysed for the tracer using an electron capture gas chromatograph. Methane can be analysed using a gas chromatograph with an FID detector (see Sect. 6.8).

The emission rate of CH_4 (L hr^{-1}) is calculated from

$$QCH_4 \ = \ QSF_6 \ \frac{[CH_4]}{[SF_6]} \tag{6.1}$$

where QSF_6 is the release rate of SF_6 in litre hr^{-1}, $[CH_4]$ is the concentration of CH_4 in the gas sample and $[SF_6]$ is the concentration of SF_6 in ppm (v/v).

6.3.2 Tracer Ratio Method for Emission Measurements in Naturally Ventilated Housing

An established approach to determine emissions at herd level in naturally ventilated housings is the use of artificial tracers with known source strength (Mohn et al. 2018; Ogink et al. 2013; Schrade et al. 2012). The most frequently used tracer gas for emission measurements in livestock husbandry is SF_6, as it is chemically extremely stable and has a concentration in ambient air of only a few ppt (10^{-12} mol mol^{-1}). The most widely used dosing principle is constant SF_6 dosing, which can be implemented for continuous or semi-continuous measurements. The quantification of emissions with the tracer ratio method is based on the assumption that the tracer gas (i.e. SF_6) release sufficiently mimics the release of the target substances from surfaces (e.g. NH_3) or animals (CH_4, CO_2). The mass flow of the target gas (m_{target}, e.g. m_{CH4}) is calculated from the ratio of the background-corrected target (c_{target}) and tracer gas concentration (c_{tracer}) and the mass flow of the tracer gas (m_{tracer}) according to m_{target} = m_{tracer} x c_{target}/c_{tracer}. In contrast to CO_2 and heat balance methods, tracer ratio methods are also applicable to naturally ventilated housings with large openings and areal sources like outdoor exercise areas and open slurry storages (Schrade et al. 2012), because the mass flow of the tracer can be adapted to the dilution ratio.

The uncertainty level of tracer gas methods is highly dependent on the accuracy and distribution of the tracer gas dosing and the number and positioning of the sampling points (Calvet et al. 2013). Homogenous dispersion can best be achieved by dosing a diluted tracer gas, with similar density to ambient air, in a dosing grid next to the emitting areas or objects. Representative, preferably homogenously mixed, air samples can be collected using an air sampling grid with critical orifices. Validation experiments of the tracer ratio method have demonstrated that the technique is suitable for both areal and point emission sources and can achieve an uncertainty of less than 10% for target gas mass emissions, which is superior to other currently available methods (Mohn et al. 2018; Schrade et al. 2018). The sensitivity of the technique is mainly given by the capability for background correction, i.e. to separate and subtract emissions in the close vicinity of the housing. This is especially important for calm nights where, depending on the topographic conditions, the target gas accumulates in the nocturnal boundary layer.

A specific variant of the tracer ratio method, using two (or more) different tracer gases (e.g. SF_6, SF_5CF_3), enables the independent assessment of housing areas (Mohn et al. 2018; Schrade et al. 2018). In an experimental housing with identical, but spatially separated housing areas, this approach can be applied to quantify the reduction potential of abatement measures (e.g. flooring, feeding) under comparative measurement conditions (e.g. climate). Additionally, using two different tracers, cross-contaminations between experimental areas are readily apparent by enhanced concentrations of the tracer gas in the respective other section.

6.3.3 Application of CH₄: CO₂ Ratio

Madsen et al. (2010) proposed using the ratio of $CH_4:CO_2$ in exhaled breath to assess enteric CH_4 production in ruminants. The principle relies on knowledge of intake, energy content and heat increment of the feed ration consumed. Haque et al. (2014) applied this method using a fixed heat increment factor. Hellwing et al. (2013) has regressed open-circuit chamber measurements of Daily Methane Production (DMP) in cattle against estimates calculated using $CH_4:CO_2$ ratios and found them to be only moderately correlated ($R^2 = 0.4$), suggesting that this method is unsuitable for precision measurements.

6.4 Micrometeorological Estimates

6.4.1 Open-Path Lasers

The use of open-path lasers combined with a micrometeorological dispersion method can now be used to measure enteric CH_4 emissions from herds of animals and facilitates whole-farm CH_4 measurements across a number of pastures.

The open-path laser method for whole-farm CH_4 measurements is already in use in Canada (McGinn 2006; Flesch et al. 2005, 2007), Australia (Loh et al. 2008; McGinn et al. 2008; Denmead 2008; Tomkins et al. 2011), New Zealand (Laubach and Kelliher 2005) and China (Gao et al. 2010). Methane concentration measurements are performed using one or more tuneable infrared diode lasers mounted on a programmable and motorised scanning unit (Tomkins et al. 2011). The tuneable infrared laser diode beams to a retroreflector along a direct path, which reflects the beam back to a detector. The intensity of the received light is an indicator of the CH_4 concentration (ppm) along the path. In an optimal situation, there should be at least one path for each predominant wind direction: one path upwind (background CH_4) and multiple paths downwind (CH_4 emission) of the herd. This method assumes that the herd acts as a surface source or, when individual animals can be fitted with GPS collars, individual animals are treated as point sources. Regardless of application, the CH_4 concentration is calculated as the absorption ratio of the external absorption to internal reference-cell absorption, of the infrared laser beam as it travels along the path (Flesch et al. 2004, 2005). Continuous measurements are required for the CH_4 concentration and the environmental measurements, the latter being recorded by a weather station, i.e. atmosphere temperature, pressure, and wind direction and speed (Loh et al. 2008, 2009). Data management can be achieved statistically by merging all data, including GPS coordinates of the field (paddock) or individual animals, from a number of averaging time periods using statistical software. After integrating, WindTrax software (Thunder Beach Scientific, Nanaimo,

Canada) uses a backward Lagrangian Stochastic (bLS) model to simulate CH_4 emission (grams day^{-1} animal^{-1}), by computing the line average CH_4 concentrations with atmospheric dispersion conditions.

The data integrity of the open-path laser method will be highly dependent on environmental factors and the location of test animals. Flesch et al. (2007) described several criteria to determine data integrity using an open-path laser method. These criteria are based on wind turbulence statistics, laser light intensity, R^2 of a linear regression between received and reference waveforms, surface roughness, atmospheric stability, and the source location (surface or point source). Invalid data can be generated by misalignment of the laser, unfavourable wind directions, surface roughness or periods in which the atmospheric conditions (rain, fog, heat waves, etc.) are unsuitable for applying the model (Freibauer 2000; Laubach and Kelliher 2005; Loh et al. 2008). To optimise the positioning of the equipment, all of these meteorological and physical aspects of the experimental site must be taken into account (Flesch et al. 2007; Loh et al. 2008, 2009). Moreover, the measurement area is restricted by the length of laser paths when using a surface source approach. It is especially important to define the herd location, as uneven distribution of the herd results in miscalculations of the CH_4 concentration. Tomkins et al. (2011), comparing open-circuit respiration chambers with the open-path laser technique, reported estimated CH_4 emissions using the bLS dispersion model of 29.7 $\pm$ 3.70 g kg^{-1} dry matter intake (DMI) relative to 30.1 $\pm$ 2.19 g kg^{-1} DMI measured using open-circuit respiration chambers.

The open-path laser method does not interfere with the normal grazing behaviour of the cattle and is non-invasive. Spatial variability is taken into account in these measurements, as the method can simulate gas fluxes over a large grazing area. Moreover, the tuneable diode laser is highly sensitive and has a fast response to changes in CH_4 concentration with detection limits at a scale of parts per trillion (ppt) (McGinn 2006). The labour intensity is low, although the equipment requires continuous monitoring. This method is expensive, which reflects not only the requirement of sensitive and rapid-response instruments to analyse CH_4 concentration, but also the requirement to capture micrometeorology data. Diurnal variations due to grazing and rumination pattern, pasture composition, and individual variation need to be considered in planning experimental protocols and are dependent on the purpose of the study to prevent over- or under-estimation of the total CH_4 emission. Furthermore, DMI determination is not very accurate as these are based on predictive models using the relationship between live weight (LW) and LW gain, following assumptions of the ARC (1980).

6.5 Short-Term Measurements

While most assessments of enteric methane emissions are focused on Daily Methane Production (DMP), or the derivative: Methane Yield (MY), there is an increasing impetus to estimate the emissions of large numbers of animals in their productive

environment. This is driven both by the demand for data to establish genetic parameters for DMP and/or to verify mitigation strategies or GHG inventories. Here, this area will be discussed only briefly, as there is at present limited scope for the application of these technologies in the developing countries. For the interested reader, the area has been ably reviewed by Hegarty (2013).

6.5.1 Spot Sampling Using Lasers

Spot measurements of CH_4 in the air around cattle's mouths have been made using laser devices to provide short-term estimates of enteric CH_4 flux (Chagunda et al. 2009; Garnsworthy et al. 2012). These estimates are then extrapolated to represent DMP. Chagunda and Yan (2011) have claimed correlations of 0.7 between laser and respiratory chamber measurements, but this claim is based on the laser apparatus measuring CH_4 concentrations in the outflow of respiratory chambers, rather than from the animals themselves; thus, this "method" remains unproven under realistic field conditions.

6.6 Indirect Methods

The procedures described below are "indirect" methods for approximating CH_4 production and do not measure levels of CH_4 per se that are produced by ruminant animals. Therefore, they should be used only if more precise measurements are logistically unachievable (e.g. for regional or country-wide surveys), or there is a need to fill gaps in a much larger survey, or obtain preliminary data before embarking on more extensive studies using more accurate direct techniques.

6.6.1 Methane Emissions from Feed and Feed Characteristics

Enteric methane production (EMP) can be estimated from intake and diet quality (digestibility), and there are a number of extant algorithms that attempt to quantify this, although it has been demonstrated that estimates of emissions can vary by 35% or more for a particular diet (Tomkins et al. 2011). Diet quality (i.e. digestibility) can be inferred from the analysis of representative samples of the rations or pasture consumed, but where intake is not measured, estimation of EMP faces considerable challenges. Models which estimate intake based on diet quality or particular feed fractions assume ad libitum access, and in situations where animals are corralled without access to feed overnight (as is frequently the case in developing countries), the validity of this assumption is likely violated (Hendricksen and Minson 1980; Jamieson and Hodgson 1979). In such cases, intake can be inferred

from energy requirements (Energy for LW flux; maintenance+lactation and pregnancy+locomotion) using published estimates such as the Nutrient Requirements of domestic ruminants (CSIRO 2007) to convert physical values to energy values and so infer intake of the estimated diet. However, the inherent variability in feed composition, intake patterns of fermentation and feed partitioning, makes precise prediction of CH_4 production from feed characteristics problematic, though there are many examples in the literature of attempting to do so.

Blaxter and Clapperton's (1965) landmark review of experimental results from measuring CH_4 generation in cattle and sheep indicates that both intake and digestibility determine the amounts of methane generated and do so in an interrelated manner. However, based on several hundred animal measurements over a range of diets, they proposed the following equation:

$$CH_4(\text{kcal}/100\,\text{kcal GE}) = 1.30 + 0.112D + L(2.37 - 0.05D) \qquad (6.2)$$

where

D = digestibility of gross energy, and L = level of intake relative to maintenance.

Moe and Tyrell (1979) and Holter and Young (1992) both developed more complex equations using carbohydrate fractions to predict CH_4 generation in dairy cattle:

$$CH_4\left(\text{MJ d}^{-1}\right) = 3.406 + 0.510\,\text{soluble residue (kg fed)} + 1.736\,\text{hemicellulose (kg fed)}$$
$$+ 2.648\,\text{cellulose (kg fed)} \qquad (6.3)$$

The ability of each of these equations to accurately predict methane generation has been assessed by Wilkerson et al. (1995). Using data from 602 cattle, they concluded that all equations showed moderate to large errors of prediction, with Blaxter and Clapperton (1965) and Moe and Tyrell's (1979) equations having the least error. Contrary to the conclusions of Wilkerson et al. (1995), Benchaar et al. (1998), in reanalysing data from published studies, concluded that the equations of both Blaxter and Clapperton (1965) and Moe and Tyrell (1979) had coefficients of determination of less than 0.6, with high errors of prediction.

Recently, an alternative algorithm for Methane Production Rate (MPR-CH_4 g d^{-1}) for cattle consuming tropical forages has been proposed by Charmley et al. (2016) to address the lack of data for estimating EMP for ruminants grazing in tropical systems.

Estimations of CH_4 production from feed characteristics alone should at best be considered as being preliminary calculations, to only be used prior to the start of proper measurements; however, a methodology based on IPCC Tier II enteric CH_4 estimation promises more accurate estimates of DMP of ruminants under field conditions. The general approach, which was developed specifically for smallholder farming systems in Africa (Goopy et al. 2018), uses total metabolic energy requirements (MERtotal) of individual cattle on a seasonal basis calculated by summing

the estimated MER for maintenance (MERM), LW gain or loss (MERG/L), lactation (MERL) and locomotion/traction(MERT). Intake can be inferred as a function of MERtotal and the weighted mean DM digestibility (DMD) of the seasonal feed baskets in the study area. DMI was used as the basis to estimate daily CH_4 production rate (MPR).

6.6.2 Emissions from Volatile Fatty Acids (VFAs)

Increasing interest in reducing emission of the important GHG methane has created a need for reliable indicators of daily methane production by ruminants without resorting to lengthy and intensive calorimetry studies. Feed intake explains the majority of differences seen in DMP. However, difficulty in assessing feed intake by grazing animals limits the application of this predictor and thus, a predictor of daily CH_4 production, which could be measured in the field, was sought. Enteric methane production is a direct function of the net amount of H_2 liberated through fermentation of feed, and there is an extensive literature which relates in vitro total fermentation to total gas production (e.g. Menke et al. 1979), and to CH_4 production in particular (i.e. Demeyer and Van Nevel 1975). Stoichiometric relationships developed in vitro typically explain more than 95% of observed H_2 present in VFAs. Thus, it has been considered that measurement of VFA levels in one or more rumen fluid samples may give a useful prediction of CH_4 production on a given day. Isotopic studies (Leng 1970; Sharp et al. 1982; Sutton et al. 2003) relating VFA concentration to VFA production indicated that a moderately strong relationship exists, suggesting that spot measures of VFA concentration (as a proxy for daily VFAs production) may provide a useful way of estimating daily CH_4 production in the field.

However, one study comparing multiple samples of VFA taken over the day, with simultaneous enteric methane production measurements, found that the average concentration of individual or total VFAs explained less than 25% of the variance in daily CH_4 production (Robinson et al. 2010) and is, thus, a poor predictor of methane production of sheep, which have typical morning and afternoon feeding periods. Sharp et al. (1982) also found poor-to-moderate correlations between CH_4 production rate and concentrations of acetate (r = − 0.1), propionate (r = 0.63), and butyrate (r = 0.92). Sutton et al. (2003) found similar relationships to Sharp et al. (1982) between VFA production rate and VFA concentration for cattle fed a mixed diet twice daily.

The disparate response of methane production and VFA concentration to feeding may result from changes in VFA absorbance and rumen volume with feeding. Variable absorption of VFAs due to differences in factors such as pH and osmolarity that change with feeding is known (Dijkstra et al. 1993). Additionally, rumen volume would itself directly affect VFA absorption by means of changing absorptive surface area available. Increases in rumen volume with feeding level are also known (Purser and Moir 1966) and could also explain how fermentation and methane production could increase without causing a proportional increase in VFA concentration, by

simply increasing the volume of rumen water in which the VFAs were dissolved. Rumen volume has been observed to increase by 20–34% in the first hour after feeding (Stewart et al. 1958). Such an increase would serve, not only to proportionally dilute VFA concentrations, but also to increase rumen surface area by an estimated 13–21%, thereby considerably enhancing the opportunity for diffusion of VFA across the rumen wall. Thus, using spot sampled concentrations of VFA in the rumen cannot be recommended as a method for estimating DMP in ruminants.

6.6.3   In Vitro Incubations

The amount of gas released from the fermentation process and from the buffering of VFAs is related to the kinetics of the fermentation of a known amount of feedstuff (Dijkstra et al. 2005). Several systems for measuring in vitro gas production have been developed which vary considerably in complexity and sophistication. Menke et al. (1979) described a manual method using gastight syringes, which involves constant registering of the gas volume produced. More recently others have described a system using pressure transducers (Pell and Schofield 1993; Theodorou et al. 1994; Cone et al. 1996). Variants now available as proprietary systems (RF, ANKOM Technology) use radio frequency pressure sensor modules that communicate with a computer interface and dedicated software which records gas pressure values.

The basic principle of the in vitro technique relies on the incubation of rumen inoculum with a feed substrate under an anaerobic environment in gastight culture bottles. Gas accumulates throughout the fermentation process, and a cumulative volume is recorded with gas volume curves being generated over time. To estimate kinetic parameters of total gas production, gas production values are corrected for the amount of gas produced in a blank incubation, and these values can be fitted with time using a non-linear curve fitting procedure in GenStat (Payne et al. 2011) or other suitable software. Headspace gas samples are taken to analyse the gas compositions and determine actual CH_4 concentrations, typically by gas chromatography, although a "quick and dirty" alternative has been developed–specifically a strongly basic solution such as NaOH is introduced into the vessel, which will subsequently cause the CO_2 to enter solution. The remaining gas is assumed to be CH_4.

Gas production is only one of the outputs of microbial fermentation, and the quality of the information derived can be improved by also considering substrate disappearance with concomitant production of VFAs (Blümmel et al. 2005).

There are two main forms of artificial rumen. In one form, rumen contents, freshly removed from a donor animal, are incubated in vitro in batch culture, while in the other form continuous culture is established. The former type of culture is valid for a period of hours, whereas the latter culture, which represents an anaerobic system not identical to the rumen, can be sustained for days or weeks. A procedure for batch-type culture is described below, while the rumen simulation technique (RUSITEC) (Czerkawski and Breckenridge 1977) is a proven system used widely throughout the world.

Neither type of in vitro system is very good for predicting methane production in vivo. The batch culture system generally lacks pH control, and the stoichiometry of product formation cannot be guaranteed to be the same as that occurring in the donor animal. For example, lactic acid is often detected in short-term in vitro incubations but not in the animal. In the RUSITEC system, the pattern of VFAs produced is usually similar, though not identical, to the in vivo situation.

Nevertheless, in vitro systems have some value for comparative purposes, e.g. for measuring the effects of additives on factors that control fermentation. The continuous culture is more valuable than the batch system, because it can take into account any adaptation the microbial population makes in response to the additive. This is particularly important for additives that affect CH_4 production. Some types of material, such as chlorinated hydrocarbons, are highly effective in the short term, but the rumen microbial population adapts eventually to become insensitive to the inhibitor. Microbial additives, on the other hand, require adaptation of the population to detect an effect, a situation that does not occur in the batch system.

6.6.4 Batch Systems

A variety of types of apparatus can be used. These differ in their cost and ease of use and range from a simple conical flask to a pH and Eh-controlled fermenter. The following criteria are essential to all of the systems:

Rumen contents must be removed from the rumen just before use; anaerobic conditions must be maintained during both transfer of the sample to the incubation vessel and during the incubation. The fermentation liquid must be incubated at 39 °C and agitated sufficiently to maintain some of the ciliate protozoa in suspension. The donor animals should be fed the feed to be used during the incubations.

6.6.4.1 Incubation of Rumen Contents in Glass Syringes: A Simple Artificial Rumen

The simplest type of short-term artificial rumen consists of test tubes or flasks with rumen contents incubated at 39 °C. Anaerobic conditions are maintained by bubbling CO_2 during incubation or by providing the vessels with Bunsen valves. The latter arrangement is difficult to manipulate (addition of substances, withdrawal of samples); an important component of fermentation products (gas) cannot be quantified, and the free venting of gas can result in contamination when radioisotopes are used. The procedure described below, which was developed by Czerkawski and Breckenridge (1977), is simple, and the apparatus is inexpensive; it does not suffer from the disadvantages discussed above, and it can be readily adapted to different requirements.

Plate 6.6 Simple apparatus
for incubating small samples
of rumen contents in a water
bath
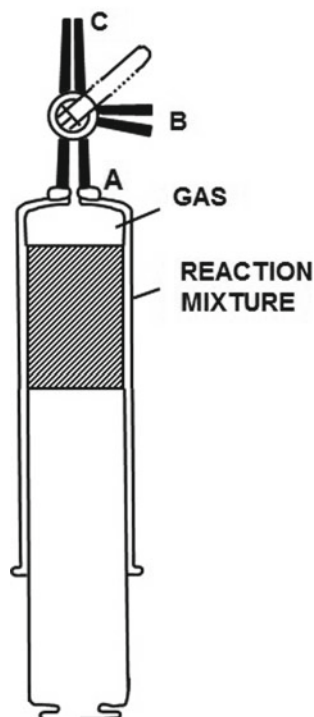
Procedure

(1) Connect a 3-way tap to a 50-ml glass syringe as shown in Plate 6.6. When the
stopcock is turned to any particular opening, that opening is closed.
(2) Fill the syringe with water and then empty it in order to wet the plunger and the
barrel. Turn the tap to C and inject 20 ml rumen contents through the opening
B, using a 20 ml syringe. Turn the tap to A and remove the 20 ml syringe.
(3) Fill a 10 ml syringe with an inert gas (e.g. N_2), connect it to B and press the
plunger, allowing the gas to escape through C until exactly 5 ml of gas is left in
the syringe. Turn the tap to C and transfer the 5 ml of gas to the bigger syringe.
Turn the tap to A and remove the small syringe. The syringe now contains 20 ml
rumen contents and 5 ml of gas which makes it possible to agitate the liquid
when the syringe is lying on its side.
(4) Place the syringe in a water bath and incubate. If a shaking water bath is available,
fix the syringes to a frame with clips. If no such bath is available, allow the
syringes to float (they will be 70–80% submerged) and remove them periodically
in order to agitate the contents by inversion at regular intervals.
(5) Take each syringe out at regular intervals, keep it vertical as shown in Plate 6.6
(making sure that the plunger is free to move) and read the volume of gas on the
scale. The difference between this reading and the amount of gas added gives
the amount of gas produced. This is not a very accurate parameter, but it is very

useful since, with care, a curve relating gas production with time can be drawn. The shape of this curve can provide useful information about the extent and magnitude of the reaction in the incubated sample.

(6) At the end of incubation, take the syringe out of the bath, connect an empty syringe (20 ml) to the opening B, turn the tap to C and transfer the gas to this syringe. If the volume of gas produced is small, use a 10 ml syringe; the measurement of gas produced will be more accurate. Turn the tap to B and disconnect the three-way tap (plus the small syringe) from the big syringe at A. Read the volume of gas and set the sealed gas sample aside for further analysis.

(7) Small amounts of liquid (e.g. substrates, labelled compounds, inhibitors) can be introduced into the reaction mixture in the same way as the gas (see step (3) above), though it is best to use a small (1 ml) plastic syringe. Similarly, samples of liquid or gas can be taken (the former, with the syringe upside down).

6.6.4.2 Continuous Fermenters

Three main types of continuous systems are used. The first is actually a sequential culture, where a small amount of solid feed is added to a sample of rumen fluid which is then sub-cultured daily (Merry et al. 1987). Whether methane production is sustained and mimics accurately, the real situation is unclear. The second type involves the continuous flow of both solid and liquid phases (Hoover et al. 1976). The third type is the RUSITEC scheme where solids are received once daily, but in the liquid phase by continuous flow. Use of the RUSITEC scheme requires a considerable input of time and expertise and is not recommended unless the specific objective is particularly suited to the apparatus, i.e. only where small quantities of the potential modifying agents are available, and the small-scale and between-vessel reproducibility offers a major advantage over animal experiments.

6.7 Methane from Animal Wastes

Animal waste is a significant source of CH_4. The amount produced depends on the diet of the animal. If the waste is stored anaerobically in lagoons or in liquid slurry tanks, CH_4 production can be closely related to the disappearance of organic matter. Temperature and relative humidity affect the rate at which the faecal matter dries out under grazing and feed conditions and determines how long fermentation continues. Estimates of CH_4 production from these systems can be obtained by adapting techniques developed for field use. The closed chamber method described for groups of animals (Chap. 2) and in rice paddies is likely to be the best method to use in measuring CH_4 production from lagoons and tanks. The emission rate of CH_4 that is likely to arise from animal waste can be very large. It may require a shorter sampling time and may also require mechanical mixing of the vented gas to prevent concentration gradients from forming in the chamber or space.

For waste lagoons, floating chambers of the types used in water studies can be employed (Chap. 2). Alternatively, modifications of the hood and facemask technique may be used, whereby air is drawn over the surface of the animal waste and subsampled for analysis. Non-isotopic tracers such as SF_6 can be used for measuring CH_4 production in barns and other enclosed areas where animal waste may accumulate.

6.8   Storage and Analysis of Samples

6.8.1   Storage of Samples

Unlike solid or liquid biological samples, which can often be frozen, dried, inactivated or preserved chemically, gas samples require very specialised handling during all stages of their analysis. Furthermore, the gases dealt with here are colourless and they have no odour. Thus, whatever the procedure, whether during sampling, storage and analysis, exceptional care should be taken to avoid losses and to eliminate any chance of such losses going undetected.

Methane passes through plastic materials, and care is required when storing gas samples prior to analysis. Glass and metal provide the most reliable materials for storing gas-containing CH_4. Methane is also relatively insoluble in water, and storage under water can provide a useful means of containing the gas.

Metal and glass syringes greased with Vaseline provide safe storage over periods of 2–3 days. These should be sealed with metal taps or hypodermic needles inserted into a rubber stopper. An important rule is that gas samples should be analysed as soon as possible after sampling; if it is necessary to store gas samples, this storage should be reduced to a minimum. Metal taps (not plastic) should be used to seal syringes. Alternatively, steel needles can be used and pushed into a rubber stopper to seal them. Bags made from PVF are satisfactory for temporary storage of gas prior to analysis, as are metal-coated liners for wine and fruit juice casks. If the aim is to store the samples for long time before analysis is carried out, then Exetainers shall be used (Plate 6.7).

6.8.2   Analysis of Samples

The methods shown in the above section require concentration measurements of CH_4, SF_6, volatile fatty acids, O_2, N_2, H_2 or CO_2. For concentration analysis, the appropriate method must be chosen under consideration of prevailing conditions, such as available apparatus, required sensitivity and the need for information about other associated gases (e.g. CO_2 or H_2). Essentially three methods are available. Whichever method is chosen, all the precautions listed above must be taken.

Plate 6.7   Exetainers for gas storage

6.8.2.1   Flammable Gases (CH$_4$ and VFA)

Flammable gases can be measured with a GC equipped with a flame ionisation detector (FID). A detailed description of this method is given in Chap. 2.

The main principle of a GC is that the gas sample (with is a mixture of several gases, e.g. O_2, CO_2, CH_4 and others like VFA or SF_6) is split into its components in a separation column. The separation columns differ in their composition, length and diameter. Make sure the column of your analytical system is appropriate for the respective gases to be measured. The manufacturer is the best source of information on appropriate columns for your measurements.

When a sample of CH_4-containing gas is injected into the GC, the gas mix is separated into its components. Depending on the retention time in the column, one compound after the next reaches the FID (CH_4 will pass through very fast). Flammable gases will trigger a signal in the detector, which is registered as a sharp peak. The area of this peak will be proportional to the concentration of CH_4.

To determine the gas concentration in your sample, it is necessary to use reference gases (i.e. gases with a known gas concentration) during all GC measurements. A detailed description of the subsequent calculations and interpretation of your samples is given in Chap. 2.

6.8.2.2   Thermal Conductivity Detector (TCD)

Gas chromatographs fitted with this type of detector come with comprehensive descriptions for use supplied by the maker, who will also supply suitable columns

and other auxiliary equipment. The use of the standard gas samples and calibration of the instrument are also dealt with by the manufacturer.

The value of this technique is that it is possible to separate all the permanent gases (O_2, N_2, H_2, CO_2, and, of course, CH_4) and to determine their relative concentrations. It is customary to use two columns, a Porapak Q column which will separate CO_2 from a composite peak containing all the other gases, and a Molecular Sieve 5A column which separates H_2, O_2, N_2 and CH_4, with CO_2 becoming part of the baseline. This means that samples have to be analysed twice. However, it is also possible to put the two columns in series on the two sides of the detector (Czerkawski and Clapperton 1968). In this method, the gas (argon) passing through one side of the detector acts as reference, and then the polarity is reversed and the other side of the detector acts as reference. It is then only necessary to inject one sample and simply to reverse the polarity of the detector during the analysis.

6.8.2.3   Infrared Detector (IRD)

An infrared gas analyser is capable of measuring CH_4 within the required range of concentrations using,

1. A flow meter.
2. Copper tubing and associated fittings.
3. A small air pump (rates as recommended by the supplier of the IR apparatus).
4. Gas driers.
5. Reference gas (N_2 if possible).
6. Standard gas of known concentration.

6.8.2.4   Laser Techniques

As described in Sect. 2.7, open-path lasers and also so-called cavity ring-down spectroscopy (CRDS) are nowadays available to determine the concentration of all relevant gases. Details on the use of these techniques can be found in Chap. 2, Sect. 2.7.

Choice of analyser: In choosing the most appropriate CH_4 analyser, it is necessary to have a suitable measurement range. In most situations, 0–500 ppm CH_4 is quite satisfactory, but by variations in analytical tube length, this range can easily be attenuated or extended. Most manufacturers can supply such options.

Of equal importance is the choice of analyser, i.e. whether it is a single channel or dual channel. In the former type, the single analytical tube is used to analyse both zero and CH_4-containing samples, but it is not possible to accommodate changes in background CH_4 concentrations or atmospheric pressure changes. In a dual-channel analyser, the use of two optically balanced analytical tubes permits the sample (unknown) gas to be measured against background air at all times in a different mode. By this procedure, changes in background CH_4 concentrations and atmospheric pressure can be fully considered, and the reliability of the resultant data increases.

Analysis: Infrared gas analysers measure CH_4 concentration in a steady stream of sample gas. Calibration procedures generally use a reference gas, i.e. N_2 or outside air if N_2 is unavailable, and a higher standard gas of known concentration. The reference and standard gases should be on either side of the range of the expected concentration of the sample gas to ensure accurate measurement of sample gas concentration. Care must be taken to ensure that the flow of sample gas to the analyser is within the recommended guidelines for the analyser and is constant throughout the analysis period. The sample gas should be dried before use. This may be accomplished by sending the gas stream through a short piece of pipe or tubing that has been loosely filled with a drying compound such as Aquasorb^{Tm} or Drierite^{Tm} or even anhydrous Na_2SO_4.

After the system is equilibrated and the reading is steady, the analyser reading is recorded and inserted into the calculation equation specific for the analyser in use.

6.8.2.5   Non-flammable Gases (CO_2 and SF6)

Concentration measurements of CO_2 and SF_6 require a GC equipped with an electron capture detector (ECD) and TCD. Note that some GCs comprise both detectors in a row so that the gas sample passes both detectors. In this case, you get gas concentration of flammable gases and non-flammable gases.

Standards should be analysed daily. A 30 ml sample of gas from a collection flask should be removed with a syringe (plastic or glass), and part of this gas is passed through the loop and the loop full of gas is injected onto the column. SF_6 will elute at approximately 20 s, and a sharp peak will be produced. O_2 has a retention time of 50 s and will be the next peak produced. It will be well resolved from the SF_6. Samples may be analysed at approximately four-minute intervals. The detection limit is approximately one part per trillion (ppt).

6.8.2.6   Standards and Calibration

In open-circuit calorimetry for measurements of CH_4 concentration and ultimately its production, it is necessary to conduct regular calibration of the CH_4 analyser and of the whole system.

(i)   *Analyser calibration*

With respect to the analyser, where an infrared apparatus is available, this should be calibrated before and after use in order to establish both zero and set point deflection and to accommodate any analyser drift. The zero-point measurement can be achieved by appropriate use of a CH_4-free air sample obtained from a fresh air source at a suitable distance from any livestock or livestock waste source. It is advisable that the gas sample is filtered and dried (e.g. through a calcium chloride tower) before injection into the analyser. A primary standard containing CH_4 can be acquired in

the first instance through a suitable commercial company or an international organisation. This standard ought to contain between 250 and 500 ppm CH_4 and should be used initially to calibrate the analyser. Once this has been achieved, gas from a larger cylinder containing a similar but unspecified concentration of CH_4 should be introduced into the analyser, and through repeated measurement its concentration should be established (secondary standard). Thereafter, this standard sample should be used regularly to standardise the analyser, and the primary standard should only be used to recalibrate new cylinders of the CH_4 standard as required.

Experience shows that often successful research work involves some improvisation. This is particularly true when one develops new methods. An example of the type of problems that can be encountered relates to efforts made by scientists in a particular institute to develop a simple method for measuring the concentrations of CH_4 in rumen gases. The apparatus required frequent calibration with standard gas mixtures. These gases were contained in two small aerosol cans and were considered to be so valuable that they were kept in a safe. During the development work, all the gas in one can was used up and it became imperative to do something before the remaining standard gas in the other can was used up since the delivery of another can would take 6–8 months. This was done by partly evacuating an old N_2 cylinder in the laboratory and carrying it to a nearby anaerobic digester on the institute farm where it was filled with CH_4-containing gas. The cylinder was then taken back to the laboratory and "topped up" with nitrogen to about 10 atmospheres, resulting in some 7% CH_4 in nitrogen. The accurate concentration of CH_4 in this secondary standard was determined using the precious primary standard. The secondary standard was then used during the remainder of the study and for many months thereafter.

Local conditions are very important. For instance, anaerobic digesters are very common in some countries, and it is easy to obtain CH_4 for the secondary standard. If there are no digesters, some other source of CH_4 has to be found; even a simple chemical method (i.e. methane derived from aluminium carbide) may be suitable.

(ii) *Whole systems*

With respect to calibration of whole systems such as respiration chambers, etc., to estimate the recovery of CH_4 and check both the analyser and non-analyser components of the system, the preferred procedure is to introduce a gas of known CH_4 concentration into the chamber over a minimum period of 6–8 h, and to determine its recovery downstream. Methane emission from the cylinder can be determined by a recording of cylinder weight loss over the test period and the CH_4 concentration of the cylinder gas, whilst quantitative recovery of CH_4 has to cover the period of time required for the concentration to return to baseline. Ideally, the recovery of CH_4 should be within $\pm 3\%$ of added methane. If the results are outside this range, the test must be repeated, and if still unsatisfactory, individual components of the system should be isolated and checked for satisfactory function.

A direct method of calibration involves weighing the CH_4 source and adding CH_4 to the chamber. CH_4 emission and analysis of outflow gas are continued until

a measurable quantity of CH_4 has been removed from the source. The source is then closed off, and analysis of outflow gas for CH_4 content continued until outflow concentration returns to original background level.

An alternative to the burning of alcohol to produce CO_2 can be simulated quite easily by acidifying a $BaCO_3$ solution with dilute hydrochloric acid within the chamber. The generation of CO_2 should be controlled by drip addition of the acid or by slow pumping of the acid to avoid massive fluctuations in CO_2 content of the outflowing air.

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