## 18.0 ESTIMATION OF METHANE EMISSION IN RUMINANTS BY SULFUR HEXAFLUORIDE TRACER TECHNIQUE

The sulfur hexafluoride ($SF_6$) tracer technique was developed at Washington State University, USA by Johnson *et al.* (1994). In this technique, a small permeation tube containing sulfur hexafluoride is placed into the rumen. $SF_6$ release rate of permeation tube is determined prior to the placement in the animal. A halter fitted with a capillary tube is placed on the animals head and connected to an evacuated canister. As the vacuum in the sampling canister slowly dissipates a steady breath sample is taken. After collection of the sample (Fig. 18.1), canister is pressurized with nitrogen gas and analyzed for methane and $SF_6$ concentration using gas chromatograph.

Methane emission rate is calculated as the product of the permeation tube emission rate and the ratio of methane to $SF_6$ in the sample.

### Equipments & parts required

- Gas chromatograph equipped with flame ionization detector (FID) and electron capture detector (ECD)
- Methane standards
- Sulfur hexafluoride standards
- Molecular sieve 5A column
- Porapack N column
- Pure sulfur hexafluoride gas
- Nitrogen gas
- Water bath
- Hot air oven
- Vacuum pump
- Pressure gauge
- Liquid nitrogen

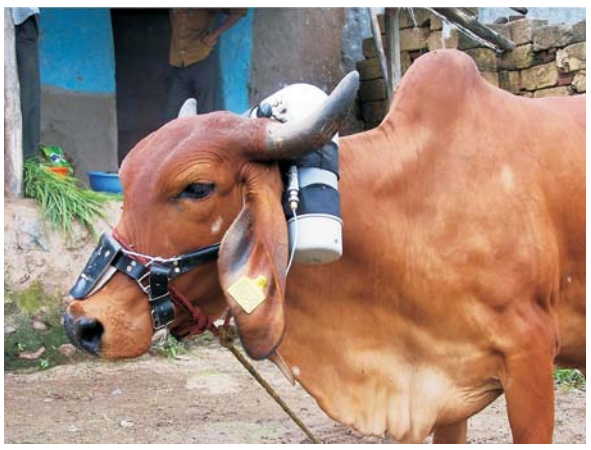

*Fig. 18.1 Collection of breath samples*

### Preparation of permeation tube & determination of $SF_6$ release rate:

### Parts required

- Brass tube body
- Teflon window
- 9/16” wrench + locking pliers
- Stainless steel frit (2µ)
- Glass receptacle in 39ºC water bath
- ¼ ” Swagelok ™ nut
- Pure $SF_6$ gas
- Liquid nitrogen
- Nitrogen gas

### Procedure

- Drill a 3/16” hole in a 1.25” x 7/16” O.D. brass rod to a depth of about 1” in one end of the rod to make $SF_6$ cavity.
- On the open end, thread the outside of the rod to allow attachment of a ¼” swage lock nut along with a thin teflon window and a stainless steel frit and put an identification at the bottom of the rod.
- Weigh the assembled empty permeation tube.

**Note:**

- The thickness and type of teflon dictate the permeation rate.
- Teflon of 12 mm thickness and a 2 µ frit will normally provide $SF_6$ permeation rates in the range of 1000-2000 ng/min at 39ºC.

**Filling of $SF_6$ in permeation tube**

- Weigh the assembled empty permeation tube (including swage lock nut, teflon window and stainless steel frit).
- Remove the nut, teflon window and frit.
- Immerse the tube body in liquid nitrogen and allow it to reach cryogen temperature.
- Remove the tube from liquid nitrogen and quickly fill with $SF_6$ and cap it with teflon window, frit and swage lock nut (before filling any liquid inside the tube is poured out of the cavity).
- Weigh the permeation tube after filling (Fig. 18.2).

*Fig. 18.2 Permeation tube*

**Determination of $SF_6$ release rate of permeation tube**

- Place the $SF_6$ filled permeation tube in a glass receptacle in a water bath (at 39ºC) and maintain a flow of clean $N_2$ gas to purge the glass receptacle of $SF_6$ emissions.
- Weigh the tubes at weekly intervals to determine the release rate of $SF_6$
- After six weeks, calculate the $SF_6$ release rate.

**Construction of breath sampling apparatus**

- Breath sampling apparatus consists of collection canister, a modified halter and capillary tubing.

**Halter construction**

- Halter size depends on the size of the animal. The size of the halter is very important, as the location of the inlet over the nostril is critical to the success of sampling (Fig. 18.3).
- Punch new holes in the halter straps for smaller animals to ensure a snug fit to the nose band.
- Rivet or sew a leather flap to the halter nose band to provide support for the capillary tube inlet.

*Fig. 18.3 Halter*

**Capillary tubing**

- The length of the capillary tubing regulates the sampling rate.
- Stainless steel tubing with an inside diameter of 0.005” and an outside diameter of 1/16” serves as the flow restrictor and transfer line.
- To determine the sampling rate of a piece of capillary tubing connect it to an evacuated canister and allow it to fill for several hours while periodically checking the pressure. Calculate the fill rate and compare it to the desired length of time.
- The canister should fill to approximately ½ atmospheric pressure after the desired collection time has completed. Filling to ½ atmospheric pressure ensures the fill rate is a linear and constant one.

- Capillary tubing has been found to be very different both within and among different lots. It is necessary to check all fill rates prior to sampling.
- After the appropriate length of capillary tubing has been selected and tested, attach a 50 micron filter to the upstream end of the capillary tube. This will also require a 1/8" to 1/16" reducing union and appropriate swage lock.
- The purpose of the filter is to protect the capillary tubing from filling with dust and debris. Attach the filter with leather noseband so that the filter and tubing on the end will be located on the above nostril of the animal.
- To the downstream end of the tubing install another 1/8" to 1/16" reducing union and connect it to 1/8" PTFE tubing. Check all fittings for fit and tightness. Run the capillary tubing up the side of the halter and tape it into the place with electrical tape. If the capillary tubing is longer than side of the halter carefully coil it up tape the coil into place.

**Parts required**

- Cow halter with adjustable chin straps
- Piece of leather with rivets
- 2.0 cm piece of tygon tubing
- 1.0 cm piece of 1/8" PTFE tubing
- 50 micron filter
- 1/8"-1/16" reducing union
- 1/16" stainless steel capillary tubing
- 1/8" PTFE tubing
- 1-male quick connect
- Assorted ferrules and swage lock nuts

**Construction of PVC collection canister**

- A PVC pipe of 2-2.5" ID and 200 psi pressure (6 kg/cm$^3$ pressure) rating is satisfactory for canister construction.
- PVC end caps (10 kg/cm$^3$ pressure) and a 90º elbow are used to seal the sample canister.
- To construct a canister, a 6 cm ID and 200 psi pressure (6 kg/cm$^3$ pressure) PVC pipe (6 kg/cm$^3$ pressure), should be cut into 31-33 cm pieces.
- Wash the inside and outside of the pipe and fittings with warm soapy water allow them to dry.
- Glue the two end PVC end caps into a 90º elbow and the end caps to the open end of the PVC pipes using PVC glue.


*Fig. 18.4 Preparation of canister*

- Wipe of excess glue and dry it for 12-24 hrs.
- Place the entire assembly into 120-135ºC ovens for 5-10 minutes. Keep checking the pipe by squeezing it until it is soft and pliable. Remove the pliable pipe and bend the legs into the desired position.
- The final dimensions of the canister depend on the size of the animal being sampled.
- For average sized cows an 8" space between the ends of the pipe is adequate. If the canister does not have the desired shape, reheat it and begin again.
- If the pipe collapses on itself it is not hot enough.

- To install gas sampling valve, tap the canister elbow, with 0.25” pipe thread. Install the gas sampling valve by gently screwing it into the threaded hole.
- A short (4”) piece of ¼” teflon tubing is attached to the valve with a female ¼” quick connect on the upstream end to allow attachment to the halter.
- Wrap the legs in tape for safety purposes against the possibility of implosion.
- After assembling, the canister (Fig. 18.4) can be checked for leaks by pressurizing it with compressed air or nitrogen to 0.4 bar and then submerging it under water.

**Parts required**

- Two 31-33 cm length pieces of PVC pipes (2.0-2.5” ID & 6 kg/cm$^3$ pressure)
- 2 end caps(10 kg/cm$^3$ pressure, 2”)
- 90$^o$ elbow
- 1 litre PVC Glue
- Hot air oven (120-135$^o$C)
- Gas sampling valve
- Swage lock fittings
- Teflon tubing
- Female quick connect
- Tape, packaging
- compressed air or nitrogen
- Water tub
- Araldite –one Tube

**Dilution system**

- A dilution system is necessary to pressurize the sample with nitrogen gas.
- In order to begin sampling, the canister should be in negative pressure, so that a steady breath sample is taken inside the canister.
- First evacuate the canister and create negative pressure by using a vacuum pump and record the pressure.
- At the time of collection, a halter fitted with a capillary tube is placed on the animals head and connected to an evacuated canister. As the vacuum in the sampling canister slowly dissipates, a steady breath sample is taken inside the canister.
- After the collection period is over, record the pressure of canister.
- Than connect the canister to the dilution system and slowly add nitrogen gas until the pressure in the canister is increased to about 1.2 atmospheric pressure and record the final pressure and calculate the dilution factor. (Sample dilution is done to bring the contents of the canister under positive pressure which enables easy transfer of an aliquot of the sample to GC systems).
- Attach canister directly to the gas sampling valve in the GC via quick connect fittings. Simply opening the canister valve will allow sample transfer to the fixed volume loop in the GC.

**Required parts**

- Nitrogen gas cylinder with regulator
- Regulator valve
- Pressure gauge
- Copper tubing
- Male quick connect
- Vacuum pump

**Methane analysis**

- Methane concentration in the sampling canister is determined by gas chromatography (Fig. 18.5).

*Fig. 18.5 Breath sample analysis using GC*

- The GC system consists of a 1 ml sampling loop attached to a low dead volume gas sampling valve, a 1/8" x 4' stainless steel packed with porapack N and flame ionization detector (FID).
- GC oven is maintained at about 50ºC and detector at 150ºC for methane analysis.
- Each analysis can be completed in less than one minute.
- Triplicate analysis should exhibit reproducibility of less than 2% or more.
- The GC oven should be conditioned to 150ºC for several hours (overnight) prior to the next analysis period.

**$SF_6$ analysis**

- $SF_6$ is measured using a gas chromatograph equipped with an electron capture detector (ECD).
- The GC system consists of a 1.0 ml sampling loop attached to a low dead volume gas sampling valve, a 1/8" x 6' stainless steel packed with molecular sieve 5A column and a electron capture detector (ECD).
- With a column temperature of 50ºC, $SF_6$ elutes in less than one minute and prior to oxygen peak.

**Calculations**

The tracer method utilizes $SF_6$ to account for dilution as gases exiting the cow`s mouth mixed with ambient air. It is assumed that the $SF_6$ emission exactly stimulates the $CH_4$ emission and thus the dilution rates of $SF_6$ and $CH_4$ are identical. The methane emission rate ($Q_{CH4}$) can then be calculated from measured $CH_4$ and $SF_6$ concentrations and the known release rate of $SF_6$ ($Q_{SF6}$):

$$Q_{CH4} = Q_{SF6} \times [CH_4] / [SF_6]$$

Background concentrations of $CH_4$ and $SF_6$ should be subtracted from the concentration measured in the sampling canister.

**Reference:** Johnson, K. A., Huyler, M.T., Westberg, H. H., Lamb, B. R. and Zimmerman, P.1994. Measurement of methane emissions from ruminant livestock using a $SF_6$ tracer technique. *Environmental Science and Technology*, 28:359-362.; Johnson, K. A., Westberg, H. H., Michal, J. J. and Cossalman, M. W. 2007.The $SF_6$ tracer technique: methane measurements from ruminants. In: *Measuring Methane Production from Ruminants,* (eds. Makkar, H. P. S. and Vercoe, P. E.), IAEA, Vienna, pp. 33-67.