In Vitro Procedure

Materials:

50 ml centrifuge tubes in rack
Whatman 541 filter paper
Aluminum pans
Gas-release valve stopper
Incubator or water bath
Centrifuge
Filtering apparatus with modified Buchner funnel

Reagents:

McDougall's artificial saliva (mix 4 parts McDougall's to 1 part rumen fluid).

1. 9.8 g $NaHCO_3$ (Mallinckrodt Cat # 7412, FW 84.01, CAS # 144-55-8, Sigma Cat # S-6014, FW 84.01, CAS # 144-55-8, and EM Cat # SX0320, FW 84.01, CAS # 144-55-8)/ Liter
2. 7.0 g $Na_2HPO_4 \cdot 7H_2O$ (Fisher Cat # S373-500, FW 268.07, CAS # 7782-85-6) / liter or use 3.71 g anhydrous (J.T. Baker Cat # 3828-01, FW 141.96, CAS # 7558-79-4) / liter
3. 0.57 g KCl (Mallinckrodt Cat #6838-05, FW 74.55, CAS # 7447-40-7 or EM Cat # PX1405-5, FW 74.55, CAS # 7447-40-7) / liter
4. 0.47 g NaCl (Omnipur Cat #7760, FW 58.44, CAS # 7647-14-5 or VWR Cat #BDH0286-500, FW 58.44, CAS #7647-14-5) / liter
5. 0.12 g $MgSO_4 \cdot 7H_2O$ (J.T. Baker Cat #2500-01, FW 246.47, CAS # 10034-99-8 or Mallinckrodt Cat # 6066, FW 246.47, CAS # 10034-99-8) / liter
6. 1.0 g Urea (Mallinckrodt Cat # 8642, FW 60.06, CAS # 57-13-6) / liter
7. 1 ml 4% $CaCl_2$ solution ((Anhydrous) EM Cat # CX0156-1, FW 110.99, CAS # 10043-52-4)

a. Mix the first six chemicals in 500 ml of di H_2O (39-40 °C) and stir until dissolved. Add remainder of water.
b. Before using, add in the 4% $CaCl_2$ solution.
c. Place CO_2 tubing into McDougall's solution and bubble in CO_2 gas until the pH of the McDougall's solution reads 6.8 to 7.0 at 39 °C.

Note: When using the CO_2 tanks, open the top release valve and then open the smaller valve to release CO_2 into the plastic line. After you have finished, close the top release valve and close the smaller line release valve. Failure to close BOTH valves results in emptying the tank (when the tank reads at 50 lbs. pressure, please tell one of the technicians, so a new CO_2 tank can be ordered).

Rumen Fluid Collection:

Ideally, rumen fluid should be collected from 3 to 4 animals, but in practice, most labs use two animals. Collection of rumen fluid can be by one of two methods.

> **Method 1:** Uses a hand held vacuum pump and metal strainer. The metal strainer is inserted below the haymat and with gentle vacuum collect enough rumen fluid for your run. Rumen fluid should be placed into a warm thermos and closed for transportation.
>
> **Method 2:** Sample can be collected by hand. Grab a hand full of the haymat and squeeze into 4 layers of cheesecloth. Dump fluid from collection pail or beaker into a warm thermos regularly and close the thermos to limit exposure to air.

Bring rumen fluid to lab, filter through 4 layers of cheesecloth, and place on a stir plate, stirring to limit settling.

Pepsin Solution:

> 1.96 g of 1:100,000 Pepsin (Sigma-Aldrich Cat # P7000-100G, CAS # 9001-75-6)
> 100 ml of 1 N HCl (EMD Cat # HX0603-3, FW 36.46, CAS # 7647-01-0) (to prepare 1 N HCl add 80.4 ml HCl / liter of di H_2O) Add di H_2O to reach 1 liter.

Procedure:

1. Weigh out a 0.25 g sample and place into a labeled 50 ml centrifuge tube (in triplicate). Label and weigh aluminum pans and dried filter paper.
2. Also, include at least 9 blanks (tubes containing no sample and 24 ml of the McDougall's with 6 ml rumen fluid mixture). Include 0.25 g samples of lab standards (use three lab standards; weigh out in triplicate).
3. Add 24 ml of the McDougall's solution to the tube and place in incubator.
4. Collect and prepare rumen fluid as above and add 6 ml rumen fluid to tubes.

Note: Amount of McDougall's and rumen fluid can be altered, but the 4:1 ratio must be maintained. **McDougall's solution and rumen fluid may be mixed and added to tubes all at once.**

5. Insert CO_2 hose above sample and flush tube with CO_2 (gently, so sample isn't disturbed). Place gas-release valve stopper on tube, invert several times to suspend the sample, and then place tubes into a rack and place the rack into a 39 °C incubator.
6. Incubate the tubes for 48 hours.
7. Swirl the tubes at 2, 4, 20, and 28 hours after initiation of incubation to suspend the sample.
8. After 48 hours of incubation, remove the tubes from the incubator. Centrifuge for 15 minutes at 2,000 rpm and aspirate off the liquid by vacuum (at this point, samples may be frozen until the pepsin digestion can be completed).
9. Make up the pepsin solution and add 35 ml of pepsin solution to each tube. Invert tubes to suspend the sample. Incubate for 24 hour in a 39 °C incubator, inverting tubes at 2, 4, and 6 hours after pepsin addition.
10. After the completion of the digestion, filter the samples using the modified Buchner funnel and pre-weighed ashless filter paper (541 filter paper). If IVOMD is not done, Whatman #54 filter paper (hardened but not ashless) may be used. It is much less expensive.

11. Dry the filter paper containing the sample in an aluminum pan for 12 to 24 hours. Record weights.
12. If OMD is needed, ash each sample and record the weights.

Calculations:

$$IVDMD = 100 \times Dry\ Wt. - \frac{(Dry\ residue\ Wt. - Blank)}{Dry\ Wt.}$$

$$IVOMD = 100 \times OM\ Wt. - \frac{(OM\ residue\ Wt. - OM\ Blank)}{OM\ Wt.}$$

$$Sample\ omf = organic\ matter\ factor, dmb = 100 - Ash\ \%, dmb$$

$$OM\ residue\ Wt. = Dry\ residue\ Wt. - [(Ashed\ residue + Pan\ Wt.) - Pan\ Wt.]$$

References

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