Buffer, Inoculum and Sample Preparation

Buffer Preparation: Kansas State Buffer (see ref. 5)

Solution A	**grams / liter**
KH_2PO_4	10.0
$MgSO_4 \cdot 7\ H_2O$	0.5
NaCl	0.5
$CaCl_2 \cdot 2\ H_2O$	0.1
Urea (optional)	0.5

Solution B	**grams / 100 ml**
Na_2CO_3	15.0
$Na_2S \cdot 9\ H_2O$	1.0

Mix 20 ml of Solution B with 1000 ml of Solution A and adjust pH to 6.8 by adding Solution B before each use.

Buffer Preparation: Goering - Van Soest Buffer (see ref. 3)

In vitro rumen buffer solution

Distilled water	1 liter
NH_4HCO_3	4 g
$NaHCO_3$	35 g
Resaruzin 0.1% (w/v)	
Dissolve 0.1 g resaruzin into 100 ml dH_20	

In vitro rumen macromineral solution

Distilled water	1 liter
Na_2HPO_4 anhydrous	5.7 g
KH_2PO_4 anhydrous	6.2 g
$MgSO_4 \cdot 7\ H_2O$	.6 g

In vitro micromineral solution

$CaCl_2 \cdot 2\ H_2O$	13.2 g
$MnCl_2 \cdot 4H_20$	10.0 g
$CoCl_2 \cdot 6\ H_2O$	1.0 g
$FeCl_3 \cdot 6\ H_2O$	8.0 g

Reducing solution

Cysteine solution	625.0 mg
1N NaOH	4.0 ml
Distilled Water	95 ml
$Na_2S \cdot 9H_2O$	625.0 mg

Add to volumetric and bring volume to 100 ml with distilled water

Buffer Preparation: Cone's Buffer / Mineral Solution (see ref. 4)

	grams / liter
$NaHCO_3$	8.75
NH_4HCO_3	1.00
Na_2HPO_4	1.43
KH_2PO_4	1.55
$MgSO_4 \cdot 7H_2O$	0.15
Na_2S	0.52
$CaCl_2 \cdot 2\ H_2O$	0.017
$MnCl_2 \cdot 4\ H_2O$	0.015
$CoCl_2 \cdot 6\ H_2O$	0.002
$FeCl_3 \cdot 6\ H_2O$	0.012
Resazurin (optional)	0.125

Buffer Preparation Procedure

(1) Maintain all glassware at 39°C.

(2) Mix enough buffer solution to support planned number of Gas Production Modules. Each Module's Glass Bottle can contain up to 200 ml of solution. (If using the larger 500 ml or 1000 ml Glass Bottle, make calculations using appropriate ratios). A smaller amount may be used to provide larger head space and accommodate a larger sample size.

(3) Prepare and pre-warm your buffer solutions. To analyze 20 samples using the buffer formula provided, combine ~340 ml of solution B to 1600 ml of solution A (1:5 ratio). The exact amount of A to B should be adjusted to obtain a final pH of 6.8 at 39°C. No further adjustment of pH is necessary.

(4) Add 100 ml of combined pH corrected solution to each Module's Glass Bottle and allow temperature of Glass Bottle and buffer to equilibrate for 20 to 30 minutes at 39°C.

(5) Add sample to Module's Glass Bottle and allow to equilibrate with buffer solution.

(6) While the buffer is equilibrating prepare the rumen inoculums.

Inoculum Preparation Procedure

(1) Preheat two 2L thermos bottles by filling with 39°C water.

(2) Empty heated water just prior to collection of rumen inoculum.

(3) Using the appropriate collection procedure, remove 600 to 1,000 ml of rumen inoculum and place in thermos. Include approximately two "fistfuls" of the fibrous mat from the rumen with your collection in one thermos.

(4) Empty the rumen inoculum and fibrous mat from the thermoses into a blender.

(5) Purge the blender container with CO_2 gas and blend at a high speed for 30 seconds. The blending action serves to dislodge microbes that are attached to the mat and assure a representative microbial population for the fermentation.

(6) Filter the blended digesta through 4 layers of cheesecloth into a pre-heated (39°C) flask. NOTE: Allow for extra cheesecloth around the edges to facilitate squeezing contents of filtered mat.

(7) The flask should be continually purged with CO2 before and after the transfer of the inoculum.

(8) Be careful to maintain temperature.

(9) Add the 25 ml of inoculum to the equilibrated buffer solution and sample in each Module's Glass Bottle.

(10) Purge the Glass Bottle with CO_2 gas for 30 seconds. A purge system can be purchased from ANKOM Technology which allows the purging to be accomplished in a closed Module. If you don't have an ANKOM purge system then remove the Glass Bottle and allow CO_2 to flow into it.

(11) Repeat process for all Glass Bottles to be used.

NOTE: Do not allow CO_2 gas to bubble through the buffered inoculum. Instead, use the CO_2 to form a gaseous blanket over the contents of the Glass Bottle.

Sample Preparation

The quantity of sample (substrate) to add to the Module will vary from 1g for a forage sample with minimal fermentable substrate to .25 g for a highly fermentable substrate such as a high starch diet. In addition to the quantity of fermentable substrate, the length of time the incubation is conducted must be taken into consideration. The quantities of substrate and buffer may be sufficient for 24 hour incubation but may not be sufficient for a 48 hour incubation. By measuring the pH at the end of the incubation period you can determine if the buffer maintained the proper pH throughout the incubation. This will allow you to alter the substrate-to-sample ration to fit the desired incubation period.

With the samples, buffer and rumen inoculum in place, be sure the digestion jars (Glass Bottles) are maintained at temperature (39o C) over the time desired for the data capture.

References

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2. Pell, A.N., Pitt, R.E., Doane, P.H., and Schofield, P., 1998, The development, use and application of gas production technique at Cornell University, USA, p.45.
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