322. METHANOSPHERA (MCB-3) MEDIUM

Rumen fluid, clarified (see medium 1310) 100.00 ml
Trypticase peptone (BD BBL) 2.00 g
Yeast extract 2.00 g
Na-acetate 0.50 g
Na-formate 0.50 g
Trace element solution (see medium 141) 10.00 ml
Na$_2$SeO$_4$ solution (0.1% w/v) 1.90 ml
NiCl$_2$ x 6 H$_2$O solution (0.1% w/v) 0.70 ml
FeSO$_4$ x 7 H$_2$O solution (0.1% w/v in 0.1 N H$_2$SO$_4$) 3.00 ml
K$_2$HPO$_4$ 0.60 g
KH$_2$PO$_4$ 2.80 g
(NH$_4$)$_2$SO$_4$ 0.30 g
NH$_4$Cl 1.00 g
NaCl 0.60 g
MgSO$_4$ x 7 H$_2$O 0.15 g
CaCl$_2$ x 2 H$_2$O 0.08 g
Na-resazurin solution (0.1% w/v) 0.50 ml
NaHCO$_3$ 4.00 g
Methanol 5.00 ml
Vitamin solution (see medium 141) 20.00 ml
L-Cysteine-HCl x H$_2$O 0.50 g
Na$_2$S x 9 H$_2$O 0.50 g
Distilled water 900.00 ml

Dissolve ingredients except bicarbonate, methanol, cysteine and sulfide, then sparge medium with 80% H$_2$ and 20% CO$_2$ gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH of medium to 6.8 - 7.0, then dispense medium under 80% H$_2$ and 20% CO$_2$ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add methanol, vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N$_2$ gas. Prior to use check pH of complete medium and adjust to 6.7 - 6.9, if necessary. After inoculation add sterile 80% H$_2$ and 20% CO$_2$ gas mixture to 1 bar overpressure.