825a. METHANOBACTERIUM II MEDIUM (N2/CO2)

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.10	g
NaCl	0.60	g
Trace element solution (see medium 141)	10.00	ml
Na-acetate	0.50	g
Yeast extract	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
Methanol	1.50	ml
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, methanol, vitamins, cysteine and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH to 7.0, then dispense medium under 80% N_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 6.8 - 7.0, if necessary.

For DSM 10111 supplement medium after autoclaving with 3.00 g/l Na-formate added from a sterile anoxic stock solution prepared under 100% N_2 gas.

For DSM 11106 supplement medium after autoclaving with 1.00 g/l Trypticase peptone and 3.00 g/l Na-formate added from sterile anoxic stock solutions prepared under 100% N_2 gas.