1084. METHERMICOCCUS (METHANOGEN) MEDIUM

KCl	0.34	g
NH_4Cl	0.25	g
K_2HPO_4	0.20	g
NaCl	24.00	g
$MgCl_2$ x 6 H_2O	10.20	g
Yeast extract	2.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
$NaHCO_3$	2.50	g
Sludge fluid (see medium 119)	5.00	ml
Methanol	8.00	ml
2-Mercaptoethanesulfonic acid (coenzyme M)	2.50	g
L-Cysteine-HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, sludge fluid, methanol, coenzyme M and reducing agents), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add sludge fluid, methanol, coenzyme M (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 6.0 - 6.5, if necessary.