1257. METHANOCELLA MEDIUM

NaCl	1.00	g
$MgCl_2$ x 6 H_2O	0.40	g
$CaCl_2$ x 2 H_2O	0.10	g
NH_4Cl	0.10	g
KH_2PO_4	0.20	g
KCl	0.50	g
Trace element solution (see medium 318)	1.00	ml
Yeast extract (OXOID)	0.10	g
Na-acetate	0.10	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Vitamin solution (see medium 141)	10.00	ml
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 carbonate, vitamins and reducing agents), then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic and adjust pH to 6.0. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add vitamins, cysteine and sulfide from sterile anoxic stock solution prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins are sterilized by filtration. If necessary, adjust the pH of the complete medium to 7.0. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.