1318. METHANOCELLA CONRADII MEDIUM

MgCl₂ x 6 H₂O 0.40 g  
CaCl₂ x 2 H₂O 0.10 g  
NH₄Cl 0.10 g  
KH₂PO₄ 0.20 g  
KCl 0.50 g  
Yeast extract 0.20 g  
Na-acetate 0.09 g  
Trace element solution SL-10 (see medium 320) 1.00 ml  
Selenite-tungstate solution (see medium 385) 1.00 ml  
Na-resazurin solution (0.1% w/v) 0.50 ml  
Na₂CO₃ 1.00 g  
Vitamin solution (see medium 141) 9.00 ml  
Seven vitamins solution (see medium 503) 1.00 ml  
Na₂S x 9 H₂O 0.20 g  
Distilled water 1000.00 ml

Dissolve ingredients (except carbonate, vitamins and sulfide) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serium vials and autoclave. Add vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. The pH of the complete medium should be 7.0. After inoculation pressurize vials to 1 bar overpressure with sterile 100% H₂ gas mixture.