332. METHANOCULLEUS BOURGENSE MEDIUM

NH_4Cl	1.0	g
K_2HPO_4 x 3 H_2O	0.4	g
$MgCl_2$ x 6 H_2O	0.1	g
Na-formate	5.0	g
Na-acetate	1.0	g
Trypticase peptone (BD BBL)	1.0	g
Yeast extract (OXOID)	1.0	g
Na-resazurin solution (0.1% w/v)	0.5	ml
L-Cysteine-HCl x H_2O	0.5	g
Na_2CO_3	1.5	g
Na_2S x 9 H_2O	0.2	g
Distilled water	1000.0	ml

Dissolve ingredients except cysteine, carbonate and sulfide. Sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic. Add and dissolve cysteine, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture and sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.
Note: After growth has started and the culture is becoming turbid add sterile 80% H_2 and 20% CO_2 gas mixture to 0.5 - 1 bar overpressure.