119b. METHANOMASSILIICOCCUS MEDIUM

KH_2PO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
$FeSO_4$ x 7 H_2O solution (0.1% w/v in 0.1 N H_2SO_4)	2.00	ml
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Yeast extract (OXOID)	1.00	g
Na-acetate	1.00	g
Na-formate	2.00	g
Sludge fluid (see medium 119)	50.00	ml
Fatty acid mixture (see medium 119)	20.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Methanol	1.50	ml
$NaHCO_3$	1.00	g
L-Cysteine-HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	930.00	ml

Dissolve ingredients except methanol, bicarbonate, cysteine and sulfide. Adjust pH of medium to 7.2 and sparge with 100% N_2 gas for 30 – 45 min to make it anoxic. Then dispense medium under same gas atmosphere into anoxic Hungate-type tubes to 30% v/v of their volume and autoclave. Add methanol, 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. Prior to use check pH of complete medium and adjust to 7.6, if necessary. After inoculation, add sterile 80% H_2 and 20% CO_2 gas mixture to 0.5 bar overpressure. After growth becomes visible overpressure of 80% H_2 and 20% CO_2 gas mixture can be increased to 1 bar.
Note: Use 10% (v/v) as inoculum.