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
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
$FeSO_4$ x 7 H_2O solution (0.1% w/v)	2.00	ml
Yeast extract (OXOID)	1.00	g
Na-acetate	1.00	g
Na-formate	2.00	g
Sludge fluid	50.00	ml
Fatty acid mixture	20.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Methanol (15% v/v)	10.00	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	940.00	ml

1. 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 (from 15% v/v stock solution), 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.

2. 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.

3. Note: Use 10% (v/v) as inoculum.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg

$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Sludge fluid (from medium 119)

Yeast extract	4.00	g
Sludge	1000.00	ml

Add 0.4% yeast extract to sludge from an anaerobic digester, and after gassing with nitrogen gas for a few minutes incubate it at 37°C for 24 hours. Then centrifuge the sludge at 13000 g and autoclave the resulting, clear supernatant in screw-capped vessels under nitrogen gas. The sludge fluid can be stored at 8-12°C in the dark.

Fatty acid mixture (from medium 119)

Isobutyric acid	23.00	ml
DL-2-Methylbutyric acid	27.00	ml
Valeric acid	27.00	ml
Isovaleric acid	27.00	ml
Distilled water	896.00	ml

Adjust pH to 7.5 with concentrated NaOH.

$FeSO_4$ x 7 H_2O solution (0.1% w/v) (from medium 119)

$FeSO_4$ x 7 H_2O	1.00	g
H_2SO_4 (0.1 N)	1000.00	ml

The ferrous sulfate solution is not stable and should be freshly prepared.