119. METHANOBACTERIUM 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
Yeast extract (OXOID)	1.00	g
Na-acetate	1.00	g
Na-formate	2.00	g
Sludge fluid (see below)	50.00	ml
Fatty acid mixture (see below)	20.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	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 bicarbonate, cysteine 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 bicarbonate, then dispense medium under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes and autoclave. Add cysteine and sulfide from sterile anoxic stock solutions 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.

Sludge fluid:
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 room temperature in the dark.

Continued next page

Fatty acid mixture:

Valeric acid	25.00	g
Isovaleric acid	25.00	g
2-Methylbutyric acid	25.00	g
Isobutyric acid	25.00	g
Distilled water	1000.00	ml

Adjust pH to 7.5 with conc. NaOH.

For DSM 1093 supplement medium after autoclaving with 0.50 g/l coenzyme M (2-mercaptoethanesulfonic acid) added from a filter-sterilized anoxic stock solution prepared under N_2.

For DSM 2030 adjust pH to 6.5 and add sterile 80% H_2 and 20% CO_2 gas to 2 bar overpressure after inoculation.

For DSM 6216 increase amount of Na-acetate to 3.00 g/l.

For DSM 7057 supplement medium with 2.00 g/l Na_2SO_4.

For DSM 9575 add sterile 80% H_2 and 20% CO_2 gas mixture to 2 bar overpressure after inoculation.

For DSM 15163 adjust pH of final medium to 6.0

For DSM 16632 and DSM 16643 replace sludge fluid with the same volume of clarified rumen fluid (see medium 1310) and supplement medium with 2.00 g/l Trypticase peptone and 10.00 ml/l of a vitamin solution (see medium 141).

For DSM 25824 supplement medium after autoclaving with 0.10 g/l 2-mercaptoethanesulfonic acid (coenzyme M) added from an anoxic stock solution sterilized by filtration and add sterile 80% H_2 and 20% CO_2 gas mixture to 1 bar overpressure after inoculation.

For DSM 25939 adjust pH of the complete medium to 7.2 and add sterile 80% H_2 and 20% CO_2 gas mixture to 1 bar overpressure after inoculation.

For DSM 25945 adjust pH of the complete medium to 7.4 and add sterile 80% H_2 and 20% CO_2 gas mixture to 1 bar overpressure after inoculation.