1084: METHERMICOCCUS (METHANOGEN) MEDIUM

KCl	0.34	g
NH_4Cl	0.25	g
K_2HPO_4	0.20	g
NaCl	24.00	g
$MgCl_2$ x 6 H_2O	10.20	g
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Sludge fluid	5.00	ml
Methanol (50% v/v)	16.00	ml
2-Mercaptoethanesulfonic acid (coenzyme M)	2.50	g
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, sludge fluid, methanol, coenzyme M, cysteine and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add sludge fluid, methanol (50% v/v solution), coenzyme M (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 6.0 - 6.5, if necessary.

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.