

1273. METHANOSPIRILLUM LACUNAE MEDIUM

KH ₂ PO ₄	0.14	g
NH ₄ Cl	0.54	g
MgCl ₂ x 6 H ₂ O	0.20	g
CaCl ₂ x 2 H ₂ O	0.15	g
Yeast extract (OXOID)	0.20	g
Na-acetate	0.80	g
Trace element solution (see medium 141)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	2.50	g
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins, cysteine and sulfide) and sparge medium with 80% H₂ and 20% CO₂ 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 vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of complete medium to 6.8, if necessary. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.