## Microorganisms



Main sol. 119		
KH <sub>2</sub> PO <sub>4</sub>	0.50	g
$MgSO_4 \times 7 H_2O$	0.40	g
NaCl	0.40	g
NH <sub>4</sub> Cl	0.40	g
$CaCl_2 \times 2 H_2O$	0.05	g
Trace element solution SL-10	1.00	ml
Yeast extract (OXOID)	1.00	g
Na-acetate	1.00	g
Na-formate	2.00	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O solution (0.1% w/v)	2.00	ml
Sludge fluid	50.00	ml
Fatty acid mixture	20.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	4.00	g
L-Cysteine HCl x H <sub>2</sub> O	0.50	g
$Na_2S \times 9 H_2O$	0.50	g
Distilled water	930.00	ml

1. 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, adjust pH to 6.5 and dispense medium under 80%  $H_2$  and 20%  $CO_2$  gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume 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.

2. 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.