

Main sol. J446

NaCl	3.00	g
MgCl ₂ x 6 H ₂ O	0.34	g
MgSO ₄ x 7 H ₂ O	0.42	g
KCl	0.05	g
K ₂ HPO ₄	0.14	g
NH ₄ Cl	0.25	g
CaCl ₂ x 2 H ₂ O	0.07	g
Trace mineral solution	10.00	ml
Na ₂ S ₂ O ₃ x 5 H ₂ O	1.00	g
Sulfur (powder)	3.00	g
NaHCO ₃	1.00	g
Distilled water	1000.00	ml

Mix ingredients except NaHCO₃ and sulfur, adjust pH to 7.5 with NaOH and autoclave. Filter-sterilize 8% NaHCO₃ solution and add to the medium. Steam sulfur for 3 hr on each of 3 successive days. Distribute the medium into culture vessels containing appropriate amount of sulfur (e.g., 20 ml of medium in 120 ml serum bottles containing 0.06 g sulfur) under a stream of CO₂-O₂ (93:7, v/v) gas mixture, seal with butyl rubber stoppers and readjust pH to 7.5 with NaOH. Pressurize the inoculated vessel to 100 kPa CO₂-O₂ (93:7, v/v).