Main sol. J417

NaCl	20.00	g
K_2HPO_4	0.90	g
KH_2PO_4	0.70	g
$CaCl_2$ x 2 H_2O	1.00	g
NH_4Cl	1.25	g
$MgSO_4$ x 7 H_2O	3.40	g
$MgCl_2$ x 6 H_2O	4.18	g
KCl	0.33	g
$NiCl_2$ x 6 H_2O	5.00	mg
Na_2SeO_3 x 5 H_2O	5.00	mg
$Fe_2(SO_4)_3$ x n H_2O	0.01	g
H_2WO_4	5.00	mg
$FeSO_4$ x 7 H_2O	0.02	g
Yeast extract (BD-Difco)	1.00	g
Tryptone (BD-Difco)	1.00	g
Sodium formate	0.20	g
Trace mineral solution	10.00	ml
Trace vitamins	1.00	ml
$NaHCO_3$	2.00	g
Sulfur (powder)	10.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Mix ingredients, except Trace vitamins, $NaHCO_3$ and sulfur, adjust pH to 5.5, and autoclave. Filter-sterilize Trace vitamins and 5% $NaHCO_3$ solution, and add to the medium. Steam sulfur for 3 hr on each of 3 successive days. Separately autoclave 5% Na_2S x 9H2O solution under a N_2 atmosphere. Distribute the medium into culture vessels (e.g., 20 ml of the medium in 120 ml serum bottles) containing sterilized sulfur under a stream of H_2-CO_2 (4:1, v/v) gas mixture, sparge for 5 min with the same gas mixture, and seal with butyl rubber stoppers. Prior to inoculation, add the sterilized Na_2S x 9H2O solution to the medium, and pressurize to 200 kPa H_2-CO_2 (4:1, v/v). Readjust pH to 5.5, if necessary.