377: PYROCOCCUS MEDIUM

NaCl	13.85	g
$MgSO_4$ x 7 H_2O	3.50	g
$MgCl_2$ x 6 H_2O	2.75	g
KCl	0.33	g
NaBr	0.05	g
H_3BO_3	15.00	mg
SrCl2 x 6 H_2O (0.1% w/v)	7.00	ml
$(NH_4)_2SO_4$	10.00	mg
Citric acid (0.1% w/v)	5.00	ml
KI (0.01% w/v)	0.50	ml
$CaCl_2$ x 2 H_2O	0.75	g
KH_2PO_4	0.50	g
$NiCl_2$ x 6 H_2O (0.1% w/v)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	30.00	g
Peptone (BD Bacto)	5.00	g
Yeast extract (OXOID)	1.00	g
Neutralized sulfide solution 3% (w/v)	15.00	ml
Distilled water	960.00	ml

Dissolve ingredients except for sulfur, peptone, yeast extract, and sulfide, adjust the pH to 6.5 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Distribute medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials that already contain the appropriate amount of sulfur. Sterilize the medium by autoclaving at **110°C** for 20 min. After sterilization, add peptone and yeast extract from sterile stock solutions prepared under 100% N_2 gas atmosphere and sulfide from a neutralized stock solution.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g

H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Neutralized sulfide solution 3% (w/v) (from medium 28)

Na_2S x 9 H_2O	3.00	g
Distilled water	100.00	ml

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 M H_2SO_4 drop-wise with a syringe without opening the bottle.