875. DESULFACINUM HYDROTHERMALE MEDIUM

Solution A:		
NaCl	26.00	g
$MgCl_2$ x 6 H_2O	5.60	g
$CaCl_2$ x 2 H_2O	1.40	g
$MgSO_4$ x 7 H_2O	6.80	g
NH_4Cl	0.25	g
KH_2PO_4	0.20	g
KCl	0.72	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	920.00	ml
Solution B:		
Trace element solution SL-10 (see medium 320)	1.00	ml
Solution C:		
Na_2CO_3	1.50	g
Distilled water	30.00	ml
Solution D:		
Vitamin solution (see medium 503)	1.00	ml
Solution E:		
Na-DL-lactate	2.50	g
Distilled water	10.00	ml
Solution F:		
Na_2S x 9 H_2O	0.40	g
Distilled water	10.00	ml

Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 – 45 min), then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. *Solutions B, E* and *F* are autoclaved separately under 100% N_2 gas atmosphere. *Solution C* is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. *Solution D* is prepared under 100% N_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of s*olutions B* to *F* are added to the sterile *solution A* in the sequence as indicated. Final pH of the medium should be 7.0 - 7.3.