795. STETTERIA MEDIUM

Synthetic seawater (2 x conc.) (see below)	500.00	ml
$NaHCO_3$	0.16	g
KH_2PO_4	0.50	g
Trace element solution (see medium 141)	15.00	ml
$NiCl_2$ x 6 H_2O solution (0.1% w/v)	3.00	ml
Peptone (BD Bacto)	2.00	g
Yeast extract (OXOID)	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Sulfur, powdered	10.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	500.00	ml

Dissolve ingredients except sulfur and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into serum vials (e.g., 20 ml medium per 100 ml bottle) that contain already the appropriate amount of sulfur. After closing pressurize bottles to 2 bar overpressure using 80% H_2 and 20% CO_2 gas mixture, than heat at 100°C for 1.5 hours on each of 3 successive days. Before use add sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas atmosphere. Adjust pH of complete medium to 6.0, if necessary. Incubate without shaking.

Synthetic seawater (2 x conc.):

NaCl	55.40	g
$SrCl_2$ x 6 H_2O	0.03	g
$MgSO_4$ x 7 H_20	14.00	g
KI	0.10	mg
$MgCl_2$ x 6 H_2O	11.00	g
Na_3-citrate	20.00	mg
KCl	1.30	g
$CaCl_2$ x 2 H_2O	1.50	g
NaBr	0.20	g
H_3BO_3	0.06	g
Distilled water	1000.00	ml

For DSM 25543 use medium without adding 80% H_2 and 20% CO_2 gas mixture before sterilization and adjust pH of complete medium to 6.8.