795a. LEBETIMONAS MEDIUM

Synthetic seawater (2 x conc.) (see medium 795)	500.00	ml
NH_4Cl	1.25	g
KH_2PO_4	0.50	g
$NiCl_2$ x 6 H_2O solution (0.1% w/v)	3.00	ml
Trace element solution (see medium 141)	10.00	ml
Na-formate	0.20	g
Tryptone (BD Bacto)	1.00	g
Yeast extract (OXOID)	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
$NaHCO_3$	2.00	g
Vitamin solution (see medium 141)	10.00	ml
Sulfur, powdered	10.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	500.00	ml

Dissolve ingredients (except bicarbonate, vitamins, sulfur and sulfide), then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic and adjust pH to 5.5 with H_2SO_4. Dispense under same gas atmosphere in suitable culture vessels (e.g. 20 ml of the medium in 100 ml serum bottles) and autoclave. Steam sulfur for 3 hours on each of 3 successive days. Aseptically mix the sterilized sulfur with the sterile medium while retaining anoxic conditions. Add vitamins from an anoxic stock solution prepared under 100% N_2 gas atmosphere and sterilized by filtration and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to inoculation add sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas atmosphere. Check pH and adjust to 5.5, if necessary. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.