770. SULFOPHOBOCOCCUS ZILLIGII MEDIUM

Solution A:		
Na_2-EDTA	32.0	mg
$CaCl_2$ x 2 H_2O	66.0	mg
$MgSO_4$ x 7 H_2O	31.0	mg
KCl	31.0	mg
$ZnCl_2$ solution (0.1% w/v)	2.1	ml
$MnSO_4$ x H_2O solution (0.1% w/v)	2.3	ml
$Na_2B_4O_7$ x 10 H_2O solution (0.1% w/v)	1.8	ml
Glycine	1.5	g
Na-resazurin solution (0.1% w/v)	0.5	ml
Na_2CO_3	230.0	mg
Distilled water	965.0	ml
Solution B:		
Yeast extract	1.0	g
Distilled water	10.0	ml
Solution C:		
Trypticase peptone (BD BBL)	1.0	g
Distilled water	10.0	ml
Solution D:		
$FeSO_4$ x 7 H_2O	50.0	mg
0.1 N H_2SO_4	5.0	ml
Solution E:		
Dithiothreitol (DTT)	150.0	mg
Distilled water	10.0	ml

Dissolve ingredients of *solution A* except carbonate, sparge with 100% N_2 gas for 30 – 45 min to make it anoxic, then add carbonate and adjust pH to 9.0 – 9.3. Dispense under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 30 ml medium per 100 ml bottle) and autoclave. *Solutions B* and *C* are autoclaved separately under 100% N_2 gas atmosphere. *Solutions D* and *E* are prepared under 100% N_2 gas and sterilized by filtration. To complete the medium add appropriate amounts of *solutions B* to *E* to the sterile *solution A* in the sequence as indicated. Adjust pH of complete medium to 7.5.
Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N_2 and filter-sterilized) may stimulate growth at the beginning. For transfers use 10% (v/v) inoculum.