1597. METHANOSALSUM NATRONOPHILUM MEDIUM

Solution A:		
Na_2CO_3	71.25	g
$NaHCO_3$	11.25	g
NaCl	4.00	g
K_2HPO_4	0.75	g
Distilled water	750.00	ml
Solution B:		
NaCl	30.00	g
K_2HPO_4	0.25	g
KCl	0.25	g
Distilled water	250.00	ml
Solution C:		
Trace elements solution (see medium 1369)	1.00	ml
Solution D:		
Selenite-tungstate solution (see medium 385)	1.00	ml
Solution E:		
NH_4Cl	0.20	g
$MgSO_4$ x 7 H_2O	0.25	g
Distilled water	5.00	ml
Solution F:		
Methanol	3.00	ml
Solution G:		
Yeast extract	0.02	g
Distilled water	5.00	ml
Solution H:		
2-Mercaptoethanesulfonic acid (coenzyme M)	0.15	g
Distilled water	10.00	ml

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Solution I:

Vitamin solution (see medium 141)	10.00	ml

Solution J:

Na_2S x 9 H_2O	0.25	g
Distilled water	10.00	ml

Sterilize *solutions A* and *B* in closed thick-walled screw-top bottles (e.g., SCHOTT) for 20 min at 120°C. The pH of *solution A* after sterilization should be 10. There is some precipitate forming that settles at the bottom after 3 – 4 days. It is best to remove precipitates by decantation before using *solution A* for medium preparation.
Combine *solution A* with *solution B* and sparge medium with 100% N_2 gas for at least 30 – 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. *Solutions C* to *J* are sterilized separately under 100% N_2 gas. Vitamins and coenzyme M should be sterilized by filtration. To complete the medium appropriate amounts of s*olutions C* to *J* are added to the combined sterile solutions *A* and *B* in the sequence as indicated. Final pH of the medium should be 9.5.
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 5 - 10% inoculum.