334a. METHANOTHRIX SOEHNGENII MEDIUM

Solution A:		
KH_2PO_4	0.22	g
Na_2HPO_4 x 2 H_2O	0.86	g
Na-acetate	6.80	g
Na_2-EDTA solution (0.1% w/v)	1.00	ml
Trace elements solution (see medium 334)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Cell-free culture supernatant of *S. associata* (see below)	100.00	ml
Distilled water	850.00	ml
Solution B:		
Na_2CO_3	1.25	g
Distilled water	25.00	ml
Solution C:		
NaCl	0.30	g
$CaCl_2$ x 2 H_2O	0.11	g
$MgCl_2$ x 6 H_2O	0.10	g
Distilled water	10.00	ml
Solution D:		
NH_4Cl	0.30	g
Distilled water	10.00	ml
Solution E:		
Vitamins solution (see medium 141)	10.00	ml
Solution F:		
Butyl vinyl ether (ALDRICH 110299)	5.00	mg
Methanol	1.00	ml
Solution G:		
L-α-Phosphatidylcholine (SIGMA P3556)	5.00	mg
Methanol	1.00	ml
Solution H:		
Na_2S x 9 H_2O	0.50	g
Distilled water	10.00	ml

Continued next page

Sparge *solution A* with 80% N_2 and 20% CO_2 gas mixture for 30 – 45 min to make it

anoxic, dispense under same gas atmosphere into anoxic serum vials or balch tubes (e.g., 20 ml medium in 50 ml bottles) and autoclave. Prior to inoculation complete the medium by adding sterile *solutions C - H* prepared under 100% N_2 gas and *solution B* prepared under 80% N_2 and 20% CO_2 gas atmosphere. Solutions *E*, *F* and *G* are sterilized by filtration. Adjust the pH of the complete medium to 7.2.
Notes: Medium is only stable for a few weeks and has to be prepared freshly. Use 20% (v/v) inoculum. Sodium sulfide should be as pure as possible (use only clean crystals for the preparation of stock solutions). It has been noted that impurities of sulfide stock solutions can inhibit growth.

Cell-free culture supernatant of Sphaerochaeta associata *DSM 26261^T*:
Cultivate DSM 26261^T in DSM medium 119 supplemented with 1.00 g/l glucose at 28°C until stationary phase is reached (7-10 days). Centrifuge culture at 3500 *xg* for 30 min, then sterilize supernatant by filtration and add it to the anoxic *solution A* before autoclaving.
Note: It is also possible to add 50.00 ml/l of a grown S. associata *culture to* solution A *before autoclaving, provided bacterial DNA from dead cells is not a problem in the downstream processing of the Methanothrix culture.*

For DSM 3870, DSM 4774 and DSM 6194 supplement medium with 10.00 ml/l of a coenzyme M (2-mercaptoethanesulfonate) solution (1.42% w/v, prepared under 100% N_2 gas and sterilized by filtration) and adjust pH of complete medium to 7.0.