334a: METHANOTHRIX SOEHNGENII MEDIUM

Solution A	942.00	ml
Solution B	25.00	ml
Solution C	10.00	ml
Solution D	10.00	ml
Solution E	1.00	ml
Solution F	1.00	ml
Solution G	1.00	ml
Solution H	10.00	ml

1. 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.

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.

For DSM 3870, DSM 4774, DSM 6194: Supplement medium with 0.14 g/l coenzyme M (2-mercaptoethanesulfonate) added after autoclaving from a sterile anoxic stock solution. Adjust pH of complete medium to 7.0.

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 (0.1% w/v)	1.00	ml
Trace elements solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Cell-free culture supernatant of *S. associata*	100.00	ml
Distilled water	830.00	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

Wolin's vitamin solution (10x)	1.00	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

Trace elements solution (from medium 334)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_3$ x 6 H_2O	1.35	g
$MnCl_2$ x 4 H_2O	0.10	g
$CoCl_2$ x 6 H_2O	0.03	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.03	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.12	g
NaCl	1.00	g
Na_2SeO_3 x 5 H_2O	0.03	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Cell-free culture supernatant of *S. associata*

1. Cultivate *Sphaerochaeta associata* DSM 26261 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.

2. 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.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml