479: METHANOHALOPHILUS MEDIUM

NaCl	87.00	g
KCl	1.50	g
$MgCl_2$ x 6 H_2O	6.00	g
$CaCl_2$ x 2 H_2O	0.40	g
NH_4Cl	1.00	g
K_2HPO_4 x 3 H_2O	0.40	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Trimethylamine-HCl	2.00	g
2-Mercaptoethanesulfonic acid (coenzyme M)	0.20	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, trimethylamine, coenzyme M and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add trimethylamine, coenzyme M and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 7.0 - 7.2, if necessary.

For DSM 5219: Supplement medium with 0.50 g/l Casamino acids (DIFCO), 10.00 ml/l of fatty acid mixture (see medium 119) and 0.25 g/l L-cysteine-HCl x H_2O from sterile anoxic stock solutions prepared under 100% N_2 gas. Adjust pH of complete medium to 7.4 - 7.5 with a sterile anoxic stock solution of NaOH.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g

$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.