479. METHANOHALOPHILUS MEDIUM

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>87.00 g</td>
</tr>
<tr>
<td>KCl</td>
<td>1.50 g</td>
</tr>
<tr>
<td>MgCl₂ x 6 H₂O</td>
<td>6.00 g</td>
</tr>
<tr>
<td>CaCl₂ x 2 H₂O</td>
<td>0.40 g</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>1.00 g</td>
</tr>
<tr>
<td>K₂HPO₄ x 3 H₂O</td>
<td>0.40 g</td>
</tr>
<tr>
<td>Trace element solution (see medium 141)</td>
<td>10.00 ml</td>
</tr>
<tr>
<td>Yeast extract (OXOID)</td>
<td>2.00 g</td>
</tr>
<tr>
<td>Trypticase peptone (BD BBL)</td>
<td>2.00 g</td>
</tr>
<tr>
<td>Na-resazurin solution (0.1% w/v)</td>
<td>0.50 ml</td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>1.50 g</td>
</tr>
<tr>
<td>Trimethylamine-HCl</td>
<td>2.00 g</td>
</tr>
<tr>
<td>2-Mercaptoethanesulfonic acid (coenzyme M)</td>
<td>0.20 g</td>
</tr>
<tr>
<td>Na₂S x 9 H₂O</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>

Dissolve ingredients except carbonate, trimethylamine, coenzyme M and sulfide. Sparge medium with 80% N₂ and 20% CO₂ 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% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ 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 H₂O from sterile anoxic stock solutions prepared under 100% N₂ gas. Adjust pH of complete medium to 7.4 - 7.5 with a sterile anoxic stock solution of NaOH.