### 1058d: THIOHALOMONAS DENITRIFICANS MEDIUM

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>120.00 g</td>
</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>1.50 g</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>0.50 g</td>
</tr>
<tr>
<td>KNO$_3$</td>
<td>1.00 g</td>
</tr>
<tr>
<td><strong>Trace elements solution (Pfennig &amp; Lippert, 1966)</strong></td>
<td>1.00 ml</td>
</tr>
<tr>
<td>CaCl$_2$ x 2 H$_2$O</td>
<td>0.05 g</td>
</tr>
<tr>
<td>MgSO$_4$ x 7 H$_2$O</td>
<td>0.50 g</td>
</tr>
<tr>
<td>Na$_2$S$_2$O$_3$ x 5 H$_2$O</td>
<td>5.00 g</td>
</tr>
<tr>
<td>NaHCO$_3$</td>
<td>5.00 g</td>
</tr>
<tr>
<td><strong>Seven vitamins solution</strong></td>
<td>1.00 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>

1. Dissolve sodium chloride, potassium hydrogenphosphate, ammonium chloride and potassium nitrate, then sparge solution with 80% N$_2$ and 20% CO$_2$ gas mixture for at least 30 - 45 min to remove dissolved oxygen. Dispense solution under same gas atmosphere in vials suitable for anaerobic cultures (e.g. Balch-type tubes) to 50% volume, close vials with butyl rubber septa and autoclave. Add trace elements, calcium chloride, magnesium sulfate, thiosulfate, and vitamins from sterile anoxic stock solutions prepared under 100% N$_2$ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N$_2$ and 20% CO$_2$ gas mixture. Thiosulfate and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 7.5 - 7.8 using a sterile anoxic stock solution of sodium carbonate (5% w/v) prepared under 80% N$_2$ and 20% CO$_2$ gas atmosphere.

2. Note: Use at least 10% (v/v) as inoculum.

For **DSM 16925**: Reduce amount of thiosulfate to 1.00 g/l.

### Trace elements solution (Pfennig & Lippert, 1966) (from medium 1369)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>5.00 g</td>
</tr>
<tr>
<td>FeSO$_4$ x 7 H$_2$O</td>
<td>2.20 g</td>
</tr>
<tr>
<td>ZnSO$_4$ x 7 H$_2$O</td>
<td>0.10 g</td>
</tr>
<tr>
<td>MnCl$_2$ x 4 H$_2$O</td>
<td>0.03 g</td>
</tr>
<tr>
<td>H$_3$BO$_3$</td>
<td>0.03 g</td>
</tr>
<tr>
<td>CoCl$_2$ x 6 H$_2$O</td>
<td>0.20 g</td>
</tr>
<tr>
<td>CuCl$_2$ x 2 H$_2$O</td>
<td>0.03 g</td>
</tr>
<tr>
<td>NiCl$_2$ x 6 H$_2$O</td>
<td>0.03 g</td>
</tr>
<tr>
<td>Na$_2$MoO$_4$ x 2 H$_2$O</td>
<td>0.03 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>

pH 3.0-4.0
### Seven vitamins solution (from medium 503)

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B$_{12}$</td>
<td>100.00</td>
<td>mg</td>
</tr>
<tr>
<td>p-Aminobenzoic acid</td>
<td>80.00</td>
<td>mg</td>
</tr>
<tr>
<td>D-(+)-biotin</td>
<td>20.00</td>
<td>mg</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>200.00</td>
<td>mg</td>
</tr>
<tr>
<td>Calcium pantothenate</td>
<td>100.00</td>
<td>mg</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>300.00</td>
<td>mg</td>
</tr>
<tr>
<td>Thiamine-HCl x 2 H$_2$O</td>
<td>200.00</td>
<td>mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00</td>
<td>ml</td>
</tr>
</tbody>
</table>