1761. PFENNIG'S MEDIUM III

Solution A:
CaCl₂ x 2 H₂O 0.25 g
Distilled water 460.00 ml
Fill 10x 46ml in 100 ml screw-cap bottles. Bubble with N₂/CO₂ and autoclave at 121°C for 15 min.

Solution B:
Na₂S x 9 H₂O 2.00 g
Distilled water 135.00 ml
Prepare in a screw-cap bottle, bubble with N₂ to replace air, close tightly and autoclave.

Solution C:
NaHCO₃ 1.50 g
H₂O 50.00 ml
Bubble with CO₂ and filter sterilize into sterile, gas-tight, 100 ml screw-cap bottle.

Solution D:
Resazurin (0.1%) 0.5 ml
Distilled water 450.00 ml

Autoclave in a cotton-stoppered Erlenmeyer flask with an outlet tube for medium, connected to a glass outlet at the bottom of the vessel and has, at the other end, a silicon rubber tube with a pinch cock and a bell for aseptic dispensing of the medium into bottles. Cool to room temperature under an atmosphere of N₂/CO₂ in an ice bath (10-15 min).

Solution E:
Ammonium chloride 0.40 g
MgSO₄x7H₂O 0.50 g
KCL 0.34 g
KH₂PO₄ 0.34 g
Trace element solution SL-12 B 1.00 ml
Distilled water 25 ml
Filter sterilize into sterile, gas-tight, 100 ml screw-cap bottle.

Solution F:
Vitamin B₁₂ 0.002 g
Distilled water 100.00 ml
Filter sterilized

Solution G:
Ammonium acetate 2.5 g
Magnesium acetate 2.5 g
Distilled water 100.00 ml
Autoclave at 121°C for 15 min.
**Trace element solution SL-12 B:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>1000.00 ml</td>
</tr>
<tr>
<td>Na$_2$-EDTA</td>
<td>3.00 g</td>
</tr>
<tr>
<td>FeSO$_4$ x 7 H$_2$O</td>
<td>1.10 g</td>
</tr>
<tr>
<td>CoCl$_2$ x 6 H$_2$O</td>
<td>190.00 mg</td>
</tr>
<tr>
<td>MnCl$_2$ x 2 H$_2$O</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>ZnCl$_2$</td>
<td>42.00 mg</td>
</tr>
<tr>
<td>NiCl$_2$ x 6 H$_2$O</td>
<td>24.00 mg</td>
</tr>
<tr>
<td>Na$_2$MoO$_4$ x 2 H$_2$O</td>
<td>18.00 mg</td>
</tr>
<tr>
<td>H$_3$BO$_3$</td>
<td>300.00 mg</td>
</tr>
<tr>
<td>CuCl$_2$ x 2 H$_2$O</td>
<td>2.00 mg</td>
</tr>
</tbody>
</table>

Adjust pH to 6.0.

**Mix solution D, C and E** in the cotton-stoppered Erlenmeyer flask. Bubble with CO$_2$ in an ice bath under sterile conditions (5-10 min).

Fill 50 ml of the mixed solution (C,D,E) into each 100 ml bottle of **solution A** (for a total volume of approx. 96 ml).

Before using, add **2,6 ml solution B** (Na$_2$S x 9 H$_2$O), **0.1 ml solution F** (Vitamin B$_{12}$) and **1 ml of solution G** (2,5% Ammonium-acetate and Magnesium-acetate) to each 100ml bottle.

Adjust the pH with filter-sterilised 1M Na$_2$CO$_3$ to 7.1-7.3.

Fill in sterile, N$_2$ gassed screw-cap tubes under N$_2$ gas.

**For the growth of DSM 11081 feed the culture with neutralized sulfide feeding solution regularly (approx. every 3 days).**

**Neutralized sulfide solution:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>100.00 ml</td>
</tr>
<tr>
<td>Na$_2$S x 9 H$_2$O</td>
<td>3.00 g</td>
</tr>
</tbody>
</table>

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 M H$_2$SO$_4$ drop-wise with a syringe without opening the bottle.

Appearance of a yellow colour indicates the drop of pH to about 8. The solution should be stirred continuously to avoid precipitation of elemental sulfur. The final solution should be clear and is yellow in colour.