1011: SULFURIMONAS MJ MEDIUM

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>30.00 g</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.14 g</td>
</tr>
<tr>
<td>CaCl₂ x 2 H₂O</td>
<td>0.14 g</td>
</tr>
<tr>
<td>MgSO₄ x 7 H₂O</td>
<td>3.40 g</td>
</tr>
<tr>
<td>MgCl₂ x 6 H₂O</td>
<td>4.18 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.33 g</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Fe(NH₄)₂(SO₄)₂ x 6 H₂O</td>
<td>0.01 g</td>
</tr>
</tbody>
</table>

**Modified Wolin's mineral solution**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaHCO₃</td>
<td>1.50 g</td>
</tr>
<tr>
<td>Na₂S₂O₃ x 5 H₂O</td>
<td>1.50 g</td>
</tr>
</tbody>
</table>

**Wolin's vitamin solution (10x)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>

1. Dissolve ingredients (except bicarbonate, thiosulfate and vitamins), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials up to a volume of 20% and autoclave. Add bicarbonate, thiosulfate and vitamins to the autoclaved medium from sterile anoxic stock solutions. Solutions of vitamins and thiosulfate are sterilized by filtration and stored under N₂, whereas the solution of bicarbonate is prepared under 80% N₂ and 20% CO₂ gas mixture and autoclaved. Adjust pH of complete medium to 6.7.

2. After inoculation pressurize vessels to 0.5 bar overpressure with sterile 80% N₂ and 20% CO₂ gas mixture and add sterile air in an amount that is equivalent to a volume of 20% of the headspace.

For **DSM 23290**: Supplement medium with 2.00 g/l NaNO₃. After inoculation pressurize vessels to 1 bar overpressure with sterile 80% N₂ and 20% CO₂ gas mixture. Do not add sterile air!

For **DSM 24660**: Omit thiosulfate and supplement medium with 4.00 g/l yeast extract and 4.00 g/l Trypton peptone. After autoclaving the medium is reduced with 0.30 g/l Na₂S x 9 H₂O added from a sterile anoxic stock solution (3% w/v) prepared under 100% N₂ gas and the pH adjusted to 6.5. After inoculation pressurize vessels to 1 bar overpressure with sterile 80% N₂ and 20% CO₂ gas mixture. Do not add sterile air!

For **DSM 28671**: Omit pressurizing vials with 80% N₂ and 20% CO₂ gas mixture.

For **DSM 101780, DSM 101688**: Supplement medium with 2.00 g/l KNO₃. Do not add overpressure of 80% N₂ and 20% CO₂ and do not add sterile air!

**Modified Wolin's mineral solution** (from medium 141)
Nitrilotriacetic acid 1.50 g
MgSO$_4$ x 7 H$_2$O 3.00 g
MnSO$_4$ x H$_2$O 0.50 g
NaCl 1.00 g
FeSO$_4$ x 7 H$_2$O 0.10 g
CoSO$_4$ x 7 H$_2$O 0.18 g
CaCl$_2$ x 2 H$_2$O 0.10 g
ZnSO$_4$ x 7 H$_2$O 0.18 g
CuSO$_4$ x 5 H$_2$O 0.01 g
AlK(SO$_4$)$_2$ x 12 H$_2$O 0.02 g
H$_3$BO$_3$ 0.01 g
Na$_2$MoO$_4$ x 2 H$_2$O 0.01 g
NiCl$_2$ x 6 H$_2$O 0.03 g
Na$_2$SeO$_3$ x 5 H$_2$O 0.30 mg
Na$_2$WO$_4$ x 2 H$_2$O 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.

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