Media Component Sheet: OSM01_01

Anaerobic Media OSM01_01

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#anaerobic steps are highlighted in blue

General remarks:

This is the medium recipe for OSM1.0. A medium that is suitable to grow the methanogens *Methanoccocus maripaludis* and *Methanosarcina barkeri,* and the sulphate reducing bacterium *Desulfovibrio vulgaris*.

The medium was formerly known as CCM-TGmod2014, which itself was a slight modification of the published CCM medium (Walker *et al*,2009).

This file only specifies the chemical medium recipe and preparation of the stock solutions and media solution. For the procedure of generating a usable, final anaerobic medium, follow "OSP1.0_Anaerobic Media Preparation Protocol".

PART A: General Protocol (for 1L media)*,^,#:

***needs to be used in conjunction with OSP1.0_Anaerobic Media Preparation Protocol**
^see part B for preparation of the various solutions.
#anaerobic steps are highlighted in blue

A1. Add the $NaHCO_3$ and **1 mL Resazurin,** which should be prepared as a 1000X concentrated stock solution, to the **Salt Solution.**

A2. Add 10 mL of **Trace Metal Solution**, which should be prepared as a 100X concentrated stock solution (as described below).

A3. If required, add a **Carbon Source** to the S**alt Solution**. If required, add **Optional Additions.**

A4. Follow the *OSP1.0_Anaerobic Media Preparation Protocol* to make the media anaerobic.

A5. Add 1 mL of **Vitamin Solution**, which should be prepared as 1000X concentrated stock solution (as described below), with a needle and syringe to avoid introducing any O_2 into the stock solution.

A6. Add 10 mL of **Cysteine-Stock**, which should be prepared as 100X concentrated stock solution (as described below), with a needle and syringe to avoid introducing any O_2 into the stock solution.

A7. Just prior to inoculation, add 100 µL of a 50X stock of **Na_2S Solution** into every 5 mL of medium, in the anaerobic chamber. **IMPORTANT NOTE:** The medium should only be stored without the Na_2S Solution added as described in the OSP1.0. This is because; Na_2S in the media makes it unstable for storage.

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PART B: Media Component Solutions[#]:

[#]anaerobic steps are highlighted in blue

1. Salt solution

Weigh out each salt below and dissolve fully in 978 mL of Milli-Q H_2O. If this is to be kept as a stock solution for later use, autoclave the solution prior to storage.

Name	g/L	g/mol	mol/L
NaCl	2.17	58.44	3.71E-02
$MgCl_2 * 6H_2O$	5.50	203.21	2.71E-02
$CaCl_2 * 2H_2O$	0.14	147	9.52E-04
NH_4Cl	0.50	53.49	9.35E-03
KCl	0.34	74.55	4.49E-03
K_2HPO_4	0.19	136.09	1.40E-03

2. $NaHCO_3$ (Dry component to act as buffer)

Weighed as a dry component and used as described in Part A, step 2.

Buffer	g/L medium	g/mol	mol/l (medium)
$NaHCO_3$	2.5	84.01	2.98E-02

3. Resazurin Stock Solution

(1000x concentrated stock solution of volume of your choice, note that amounts shown below are for 1L**).**

Dissolve 1 g of Resazurin powder into 1 Litre of water. To prepare less than 1 Litre, use a corresponding smaller ratio of resazurin.

Indicator	g/L Stock	g/L medium	g/mol	mol/l (medium)
Resazurin	1	1.00E-03	229.2	4.36E-06

4. Trace Metal Solution

(100x concentrated stock solution of volume of your choice, note that amounts shown below are for 1L**).**

TG-2014 Trace Metal	g/L(stock)	g/L medium	g/mol	mol/l (medium)
Nitrilotriacetic acid	1.5000	0.015000	191.14	7.8E-05
$MgCl_2 * 6 H_2O$	2.4800	0.024800	203.3	1.2E-04
$MnCl_2 * 4H_2O$	0.5854	0.005854	197.9	3.0E-05
NaCl	1.0000	0.010000	58.44	1.7E-04

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$FeCl_2 * 4 H_2O$	0.0715	0.000715	198.81	3.6E-06
$CoCl_2 * 6 H_2O$	0.1524	0.001524	237.93	6.4E-06
$CaCl_2 * 2 H_2O$	0.1000	0.001000	147.01	6.8E-06
$ZnCl_2 * 4 H_2O$	0.0853	0.000853	136.315	6.3E-06
$CuCl_2$	0.0054	0.000054	134.452	4.0E-07
$AlCl_3$	0.0103	0.000103	133.34	7.7E-07
H_3BO_3	0.0100	0.000100	61.83	1.6E-06
$Na_2MoO_4 * 2 H_2O$	0.0100	0.000100	205.92	4.9E-07
$NiCl_2 * 6 H_2O$	0.0300	0.000300	237.69	1.3E-06
$Na_2SeO_3 * 5 H_2O$	0.0003	0.000003	262.94	1.1E-08
$Na_2WO_4 * 2H_2O$	0.0080	0.000080	329.8477	2.4E-07

4.1. Dissolve Nitrilotriacetic acid
4.2. Adjust pH to 6.5 with 1 M KOH
4.3. Add all other minerals
4.4. Set final pH to 7.0 with 1 M KOH
4.5. Autoclave
4.6. Store at 4° C until usage

5. Vitamin Stock Solution – to be added during degassing!

(1000X concentrated stock solution of volume of your choice, note that amounts shown below are for 1L**).**

Pre-step: Prepare degased closed serum flasks for aliquoting (aliquoting of the total solution into smaller volumes is to make smaller batches is good practice, for better storage/ ease of use, minimize contamination, etc). In brief, crimp seal serum flasks. Add gas (15 minutes) to the bottle via a needle, with another needle as a vent. Pull needles out simultaneously, while gas is still flowing. Autoclave the sealed, degassed serum flasks.

5.1. Weigh all the Vitamins below into a 50 mL conical centrifuge tube.

Vitamins	**g/L(stock)**	**g/L medium**	**g/mol**	**mol/l (medium)**
Biotin	0.020	0.00002	244.31	8.2E-08
Folic Acid	0.020	0.00002	441.4	4.5E-08
Pyridoxin HCl	0.100	0.0001	205.63	4.9E-07
Thiamine HCl	0.050	0.00005	337.26	1.5E-07
Riboflavin	0.050	0.00005	376.36	1.3E-07
Nicotinic Acid	0.050	0.00005	123.11	4.1E-07
D-Ca-Panthotenate	0.050	0.00005	238.265	2.1E-07
p-Aminobenzoic Acid	0.050	0.00005	137.14	3.6E-07
Vitamin B12	0.001	0.000001	1355.37	7.4E-10
Lipoic Acid	0.050	0.00005	206.33	2.4E-07

5.2. Degas the centrifuge tube , containing the vitamins, and 1 L of Milli-Q H_2O in the anaerobe chamber over night.
5.3. Mix the dry Vitamins with the 1L of Milli-Q inside the anaerobe chamber.
5.4. Aliquot (equal amounts of your choice) from the 1L solution into sterile anaerobe serum flasks by 0.2 µm filtration (in the anaerobic chamber).

6. Cysteine-Stock – to be added during degassing!

(100X concentrated stock solution of volume of your choice, note that amounts shown below are for 1L**)**

Cysteine Solution	g/L(stock)	g/L medium	g/mol	mol/l (medium)
Cysteine-HCl * H_2O	35.03	0.35	175.16	2.00E-03

6.1. Fill Milli-Q H_2O and the corresponding amount of Cysteine-HCl * H_2O into two different tubes.
6.2. Leave both tubes in the anaerobe chamber overnight to degas.
6.3. Combine water and salt in one container and mix by inverting until the salt has completely dissolved.
6.4. Fill into serum flask.
6.5. Seal in anaerobic cabinet and take out.
6.6. Autoclave.

7. Na_2S Stock Solution – to be added just prior to inoculation!

(50x stock solution of volume of your choice, note that amounts shown below are for 1L).

Safety: *Sodium sulphide is strongly alkaline and can cause skin burns. Acids react with it to rapidly produce hydrogen sulphide, which is highly toxic. Hazard Groups: Corrosive ©, Dangerous for the environment (N). Steps 7.1-7.3 should be done in a fume cupboard.*

Na_2S Solution	g/L(stock)	g/L medium	g/mol	mol/l (medium)
Na_2S * 9H2O	24	0.48	240.18	2.00E-03

7.1. Weight out the required amount of Na_2S * $9H_2O$ carefully.
7.2. Wash the crystals with Milli-Q H_2O and dry on a piece of microscope paper (to avoid fibres) on top of a piece of kitchen roll.
7.3. Fill the amount of Na_2S * $9H_2O$ required into a tube and the corresponding amount of water in to a second tube.
7.4. Leave both tubes in the anaerobe chamber over night to degas.
7.5. Mix and fill into serum flask.
7.6. Seal in anaerobic cabinet and take out.
7.7. Autoclave.

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8. Carbon Source (substrate)

Following are some carbon sources and amounts we routinely use with this media. You might want to use other C-sources as well. Note that depending on the nature of the C-source, you might want to add after the degassing stage. For example, methanol, that can be used for M.barkeri, could have high volatility and be lost during degassing.

Organism	Name	g/L	g/mol	mol/L
Standard Recipe	Na-Lactate	3.36	112.06	3.00E-02
D.vulgaris	Na-Lactate	3.36	112.06	3.00E-02
M.barkeri	Na-Acetate	8.20	82.03	0.1
M.maripaludis	Na-Acetate	0.82	82.03	0.01

9. Optional additions

These are additions that are thought to improve monoculture growth.

Organism	Name	g/L	g/mol	mol/L
D.vulgaris	Na_2SO_4	3.36	142.04	1.00E-02
M.maripaludis	NaCl	4	58.44	6.84E-02
M.maripaludis	Yeast Extract	2	-	-

References:

Walker, C.B. et al., 2009. The electron transfer system of syntrophically grown Desulfovibrio vulgaris. *Journal of bacteriology*, 191(18), pp.5793–801.