# Media for dechlorinators from the Löffler lab

http://web.utk.edu/~microlab/LoefflerLab/Home.html

## Mineral Salts Medium ("DCB-1 medium")

***Protocol***

| Salts | 1 x [g/L] | 100 x stock [g/L] |
|---|---|---|
| NaCl | 1.0 | 100.0 |
| $MgCl_2$ x 6 $H_2O$ | 0.5 | 50.0 |
| $KH_2PO_4$ | 0.2 | 20.0 |
| $NH_4Cl$ | 0.3 | 30.0 |
| KCl | 0.3 | 30.0 |
| $CaCl_2$ x 2 $H_2O$ | 0.015 | 1.5 |

| To prepare medium | 1 L |
|---|---|
| 100 x salts | 10 ml |
| Trace element solution | 1 ml |
| Se/Wo solution | 1 ml |
| Resazurin (0.1% solution) | 0.25 ml |
| Electron donor | optional |
| $H_2O$ bidest. | add 1000 ml |

Some chemicals (e.g. fumarate, pyruvate) should not be autoclaved

- boil, cool down to room temperature under flushing with $N_2/CO_2$ (80/20
- add 0.2 mM L-cysteine (MW 157.6) 0.031 g/L
- add 0.2 mM $Na_2S$ x 9 $H_2O$ 0.048 g/L [or: add 1 mM DL-dithiothreitol 0.154 g/L]
- add 30 mM $NaHCO_3$ 2.52 g/L

adjust pH to 7.2 - 7.3 with $CO_2$ (to lower the pH with $CO_2$ is less time consuming)

- flush serum bottles with $N_2/CO_2$
- dispense medium (ca. 98 ml per bottle), close the bottles with black rubber stoppers (or Teflon-lined stoppers)
- autoclave after medium turned clear; autoclave bottles in a closed basket; do **NOT** remove bottles from autoclave before temperature of the medium has reached <70ºC

Electron donors (formate, acetate, lactate, propionate) and acceptors (PCE or *cis*-DCE) can be added prior to heat sterilization. Fumarate, pyruvate are heat-labile and should not be autoclaved.

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## Trace element solution

Per liter: HCl (25% solution, w/w), 10 ml; $FeCl_2$ x 4 $H_2O$, 1.5 g; $CoCl_2$ x 6 $H_2O$, 0.19 g; $MnCl_2$ x 4 $H_2O$, 0.1 g; $ZnCl_2$, 70 mg; $H_3BO_3$, 6 mg; $Na_2MoO_4$ x 2 $H_2O$, 36 mg; $NiCl_2$ x 6 $H_2O$, 24 mg; $CuCl_2$ x 2 $H_2O$, 2 mg

## Se/Wo solution

Per liter: 6 mg $Na_2SeO_3$ x 5 $H_2O$, 8 mg $Na_2WO_4$ x 2 $H_2O$ and 0.5 g NaOH

# Wolin Vitamins

Wolin, F. A., M. J. Wolin, and R. S. Wolfe. 1963. Formation of methane by bacterial extracts. J. Biol. Chem. **238:**2882-2886.

| Vitamins | 1000 x [mg/L] | Final conc. |
|---|---|---|
| biotin | 20 mg/L | 0.02 mg/L |
| folic acid | 20 mg/L | 0.02 mg/L |
| pyridoxine hydrochloride | 100 mg/L | 0.1 mg/L |
| riboflavin | 50 mg/L | 0.05 mg/L |
| thiamine | 50 mg/L | 0.05 mg/L |
| nicotinic acid | 50 mg/L | 0.05 mg/L |
| pantothenic acid | 50 mg/L | 0.05 mg/L |
| vitamin $B_{12}$ | 1 mg/L | 0.001 mg/L |
| p-aminobenzoic acid | 50 mg/L | 0.05 mg/L |
| thioctic acid | 50 mg/L | 0.05 mg/L |

adjust pH to ~7.5 with 10 M NaOH (takes some time)

aliquot in 20 ml portions, freeze, store in dark place (light sensitive)

prepare a 200 x working stock solution, filtersterilize