1681. Mineral salt medium with colloidal chitin

Solution A	50.00	ml
Solution B	50.00	ml
Trace element solution (see medium 141)	10.00	ml
Vitamin solution (see medium 141)	10.00	ml
Na-resazurin solution (0.1% w/v)	1.00	ml
Yeast extract	1.00	g
Polypeptone	10.00	g
$NaHCO_3$	3.52	g
Colloidal chitin	5.00	g
Distilled water	890.00	ml

Solution A

KH_2PO_4	8.00	g
NH_4Cl	5.00	g
$MgCl_2$ x 6 H_2O	4.00	g
$CaCl_2$ x $2H_2O$	1.00	g
Distilled water	1000	ml

Solution B

K_2HPO_4	8.00	g
Distilled water	1000	ml

Reducing agents

Flush each solution under N_2 for 30 minutes, stopper and autoclave.
L-Cysteine-HCL x H_2O solution (10%): add 12.5 µl per 10 ml medium
Na_2S x 9 H_2O solution (3%): add 40 µl per 10 ml medium.

Colloidal chitin preparation

Cut 5 gram commercial crab (shrimp) shell flakes into small pieces, and dissolve in 100 ml of 12M HCL while stirring in a fume hood at room temperature. Add chitin-HCL mixture slowly into pre-cooled water to obtain the colloidal chitin, centrifuge and wash in pre-cooled water till the colloidal chitin reaches a pH of 6 to 6.5. Mix 0.5% with MS medium as the sole carbon source. As an alternative commercial available chitin powder can be used.

Prepare MS medium with chitin

Dissolve the ingredients of MS medium (except $NaHCO_3$ and vitamins), boil medium under CO_2 until the resazurin indicator changes from pink to colourless. Cool down under CO_2, add $NaHCO_3$ and adjust pH to 7.0. Switch to N_2 to prevent further lowering of the pH. Fill up into Hungate tubes under N_2 and autoclave. Before use add vitamins (sterilized by filtration) and reducing agents.