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
NaHCO₃ 3.52 g
Colloidal chitin 5.00 g
Distilled water 890.00 ml

Solution A
KH₂PO₄ 8.00 g
NH₄Cl 5.00 g
MgCl₂ x 6 H₂O 4.00 g
CaCl₂ x 2H₂O 1.00 g
Distilled water 1000 ml

Solution B
K₂HPO₄ 8.00 g
Distilled water 1000 ml

Reducing agents
Flush each solution under N₂ for 30 minutes, stopper and autoclave.
L-Cysteine-HCL x H₂O solution (10%): add 12.5 µl per 10 ml medium
Na₂S x 9 H₂O 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 NaHCO₃ and vitamins), boil medium under CO₂ until the resazurin indicator changes from pink to colourless. Cool down under CO₂, add NaHCO₃ and adjust pH to 7.0. Switch to N₂ to prevent further lowering of the pH. Fill up into Hungate tubes under N₂ and autoclave. Before use add vitamins (sterilized by filtration) and reducing agents.