766. THERMOCOCCUS CHITINOPHAGUS MEDIUM

$(NH_4)_2SO_4$	0.500	g
$NaHCO_3$	0.200	g
KH_2PO_4	0.500	g
Na_2WO_4 x 2 H_2O	0.150	mg
Na_2SeO_4	0.150	mg
$(NH_4)_2Ni(SO_4)_2$	0.300	mg
Chitin stock solution (see below)	4.000	g
Na_2S x 9 H_2O	0.500	g
Trace elements SL-6 (see medium 141)	15.000	ml
Resazurin	0.001	g
Synthetic seawater (see medium 600)	485.000	ml
Distilled water	500.000	ml

The chitin solution is prepared as follows: mix 20 g chitin (practical grade from crab shells) with 200 ml of 37% HCl (pre-cooled to 4°C) and stir for 1 hr at 4°C. Pour the suspension into 1 litre of distilled water (pre-cooled to 4°C) and filter through filter paper (Schleicher and Schüll Nr. 311853). Wash the residue five times with 500 ml distilled water and resuspend in 1 litre of distilled water. Neutralise the suspension with 10 ml of 5M KOH (final pH 6.5). Filter and wash with 3 litres of distilled water to remove KCl.

Prepare the medium without Na_2S x 9 H_2O and $NaHCO_3$, boil and cool under N_2. Add the $NaHCO_3$ to the cooled medium and adjust the pH to 6.7. Dispense into Hungate tubes or serum bottles under N_2 and autoclave. Reduce the medium before use from a sterile stock solution of neutralised Na_2S x 9 H_2O.