1058c: THIOHALORHABDUS DENITRIFICANS MEDIUM

NaCl	180.00	g
K_2HPO_4	1.50	g
NH_4Cl	0.50	g
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
$CaCl_2$ x 2 H_2O	0.05	g
$MgSO_4$ x 7 H_2O	0.50	g
$Na_2S_2O_3$ x 5 H_2O	5.00	g
$NaHCO_3$	5.00	g
Seven vitamins solution	1.00	ml
Distilled water	1000.00	ml

1. Dissolve sodium chloride, potassium hydrogenphosphate and ammonium chloride, then sparge solution with 80% N_2 and 20% CO_2 gas mixture for at least 30 - 45 min to remove dissolved oxygen and to saturate the solution with CO_2. Dispense solution in vials suitable for anaerobic cultures (e.g. Balch-type tubes) to 30% volume under 80% N_2 and 20% CO_2 gas atmosphere. Close vials with butyl rubber septa and autoclave. Add trace elements, calcium chloride, magnesium sulfate, thiosulfate, and vitamins from sterile stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Thiosulfate and vitamins are sterilized by filtration. Adjust pH of the medium to 7.5 - 7.8 using a sterile stock solution of sodium carbonate (5% w/v).

2. After inoculation, add sterile air (with hypodermic needle through the rubber closure) to a concentration of ca. 4% (v/v) O_2 (e.g., add 7 ml of air to a Balch-type tube with a total volume of 27 ml).

3. Note: Use at least 10% (v/v) as inoculum.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

Seven vitamins solution (from medium 503)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml