ATCC medium: 2433 Rhodoferax ferrireducens medium

Basal Medium:

$NaHCO_3$.....2.5 g
NH_4Cl.....0.25 g
NaH_2PO_4 . H_2O.....0.6 g
KCl.....0.1g
Wolfe's Vitamin Solution (see below)....10.0 ml
Wolfe's Mineral Solution (see below)....10.0 ml
Distilled water.....980.0 ml

Medium should not be exposed to sunlight. Combine all basal medium ingredients.
Heat to boiling. Drive out O_2 with a gas mixture of 80% N_2, 20% CO_2 (final pH 6.8-7.0). Dispense into appropriate tubes in 10-ml aliquots. Autoclave 20 minutes at 121C.

Wolfe's Vitamins:
Available from ATCC as a sterile ready-to-use liquid (Vitamin Supplement, catalog no. MD-VS).
Folic acid.....2.0 mg
Pyridoxine hydrochloride....10.0 mg
Riboflavin.....5.0 mg
Biotin.....2.0 mg
Thiamine.....5.0 mg
Nicotinic acid.....5.0 mg
Pantothenic acid.....5.0 mg
Vitamin B12.....0.1 mg
p-Aminobenzoic acid.....5.0 mg
Thioctic acid.....5.0 mg
Distilled water.....1.0 L

Wolfe's Mineral Solution:
Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS).
Nitrilotriacetic acid.....1.5 g
$MgSO_4$. $7H_2O$ .....3.0 g
$MnSO_4$. H_2O .....0.5 g
NaCl.....1.0 g
$FeSO_4$. $7H_2O$ .....0.1 g
$CoCl_2$. $6H_2O$ .....0.1 g
$CaCl_2$.....0.1 g
$ZnSO_4$. $7H_2O$ .....0.1 g
$CuSO_4$. $5H_2O$ .....0.01 g
$AlK(SO_4)_2$. $12H_2O$.....0.01 g
H_3BO_3 .....0.01 g
Na_2MoO_4 . $2H_2O$.....0.01 g
Distilled water.....1.0 L

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.

Prepare the following stocks for delivery with the tubes of medium:

1.0 M Acetate stock solution:
Add 13.6 g sodium acetate to 80.0 ml distilled water. Bring volume to 100.0 ml.

Bubble with N_2 for 45 minutes; filter-sterilize.

500.0mM Fe(III)NTA Stock Solution:
Add 8.2 g $NaHCO_3$ to 70.0 ml distilled water.
Add 12.8 g of sodium nitrilotriacetic acid (NTA).
Add 13.5 g $FeCl_3$. $6H_2O$.

Adjust pH to 6.5 using 10 N NaOH; bring solution to final volume of 100 ml; ingredients will go into solution after stirring for 15 minutes. Bubble with anaerobic gas (N_2) for 45 minutes and then filter-sterilize (0.2-micron filter).

Dispense into a sterile, anaerobic, serum bottle.