ATCC medium: 2298 Medium for *Sterolibacterium denitrificans*

Solution A:
KH_2PO_4 .....0.4 g
K_2HPO_4 .....1.2 g
NH_4Cl .....0.3 g
NaCl.....0.5 g
KNO_3 .....1.0 g
Cholesterol.....0.386 g
Wolfe's Mineral Solution (see below).....1.0 ml
Distilled deionized water.....1.0 L

Solution B:
$MgCl_2 \cdot 6H_2O$ .....11.6 g
$CaCl_2 \cdot 2H_2O$ .....3.7 g
Distilled deionized water.....1.0 L

Solution C:
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
$MgSO_4 \cdot 7H_2O$ .....3.0 g
$MnSO_4 \cdot H_2O$ .....0.5 g
NaCl.....1.0 g
$FeSO_4 \cdot 7H_2O$ .....0.1 g
$CoCl_2 \cdot 6H_2O$ .....0.1 g
$CaCl_2$ .....0.1 g
$ZnSO_4 \cdot 7H_2O$ .....0.1 g
$CuSO_4 \cdot 5H_2O$ .....0.01 g
$AlK(SO_4)_2 \cdot 12H_2O$.....0.01 g
H_3BO_3 .....0.01 g
$Na_2MoO_4 \cdot 2H_2O$.....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.

Solution D:
$Na_2S \cdot 9H_2O$ .....25.0 g
Distilled deionized water.....1.0 L

Autoclave solution at 121C for 15 minutes.

Directions for preparations of complete medium: Prepare Solution A. Adjust pH to 6.8-7.0. Bring to a boil and cool under 70% N_2, 30% CO_2. Add 1.68 grams of $NaHCO_3$, 20.0 ml of Solution B, and 1.0 ml of Solution C. Autoclave medium at 121C for 15 minutes. Cool under same gas, add 1.0 ml of sterile Solution D. Check pH and adjust to 6.8-7.0 if necessary. Dispense aseptically and anaerobically into sterile anaerobic tubes with rubber stoppers.

Note: Different gas combinations may be used if 70:30 is not available. However, special care must be used to adjust the pH since the CO_2 and $NaHCO_3$ form a buffer system that affects the pH.