ATCC medium: 1207 BC Medium for *Acetivibrio cellulolyticus*

Mineral Solution 1 (see below).....75.0 ml
Mineral Solution 2 (see below).....75.0 ml
$FeSO_4$. $7H_2O$ Solution (see below).....10.0 ml
Vitamin mixture (see below).....10.0 ml
Wolfe's Mineral Solution (see below).....10.0 ml
$NaHCO_3$.....2.0 g
Resazurin solution (0.1% aqueous).....1.0 ml
Distilled water.....810.0 ml
Alpha-Cellulose (Sigma C-6429).....3.0 g
Cysteine-Sulfide Reducing Solution (see below).....12.8 ml

The pH should be 7.6 at room temperature; do not adjust.
Bring medium to boil under 10% CO_2 90% N_2. Cool and continue flushing. Add 8 ml reducing solution. Heat slightly to mix if necessary and add an additional 4.8 ml of reducing solution. Dispense 7.0 ml/tube (anaerobically) and cap.
A note of caution:
This medium contains sodium sulfide and hydrogen sulfide production will occur. Hydrogen sulfide is hazardous and this medium should be prepared in a chemical fume hood.

Mineral Solution 1:
K_2HPO_4 .....3.9 g
Distilled water.....1.0 L

Mineral Solution 2:
KH_2PO_4 .....2.4 g
Na_2SO_4 .....2.5 g
NH_4Cl.....12.0 g
$CaCl_2$. $2H_2O$ .....0.8 g
$MgSO_4$. $7H_2O$ .....1.2 g
Distilled water.....1.0 L

$FeSO_4$. $7H_2O$ Solution:
Dissolve 0.2 g $FeSO_4$. $7H_2O$ in 100 ml distilled water. Add 3 drops concentrated HCl.

Vitamin Mixture:

Biotin	2.0 mg
B6 (pyridoxine hydrochloride)	10.0 mg
B1 (thiamine . HCl)	5.0 mg
B12 (crystalline cyanocobalamin)	5.0 mg
p-Aminobenzoic acid	0.5 mg
Lipoic acid (D,L-6,8-Thioctic acid)	5.0 mg
Distilled water	1.0 L

Store below -20 C.

Wolfe's Mineral Solution:

Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS.)

Nitriloacetic 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.

Cysteine-Sulfide Reducing Solution (1.25%):

L-Cysteine . HCl	2.5 g
$Na_2S \cdot 9H_2O$	2.5 g
Distilled water to	200.0 ml

Dissolve cysteine in 50 ml distilled water. Adjust to pH 10 with fresh 3 N NaOH (rapidly) and start flushing with nitrogen. Add $Na_2S \cdot 9H_2O$. Bring total volume to 200 ml (add 150 ml but subtract the volume of 3 N NaOH and boil under nitrogen). Transfer anaerobically to tubes; stopper with butyl rubber and autoclave.