MXWT Medium

Adapted from Zhu et al., Appl. Environ. Microbiol. 2008, with the following modifications:

a) EDTA used at a concentration of 20 mg/L instead of 8.4 mg/L
b) $MgSO_4 \cdot 7H_2O$ used at a concentration of 0.45 g/L instead of 0.15 g/L
c) citric acid used at a concentration of 50 mg/L instead of 1.2 g/L
d) no leucine, Al, nor Ca.
e) NH_4Cl and K_2SO_4 used instead of $(NH_4)_2SO_4$

1) FOR EACH OF TWO SHAKE FLASKS

Medium for 50 mL volume:

Solution MXWT-A (prepare fresh, 40 mL needed)
(autoclaved)

KH_2PO_4	1.80 g/L (72 mg/40 mL)
$K_2HPO_4 \cdot 3H_2O$	3.175 g/L (127 mg/40 mL)
K_2SO_4	2.5 g/L (100 mg/40 mL)
NH_4Cl	4.38 g/L (175 mg/40 mL)
$Na_2(EDTA) \cdot 2H_2O$	25.0 mg/L (1 mg/40 mL)

Adjust to pH 7.0 with 30% (w/v) NaOH

Solution MXWT-B (solution may be stored on counter, 2.5 mL needed)
(autoclaved)

$MgSO_4 \cdot 7H_2O$	9.0 g/L

Solution MXWT-C (solution may be stored in refrigerator, 2.5 mL needed)
(filtered)

thiamine·HCl	0.40 g/L

Solution MXWT-D (solution may be stored on counter, 50 µL needed)
(filtered)

Citric acid	50 g/L
$ZnSO_4 \cdot 7H_2O$	0.25 g/L
$CuCl_2 \cdot 2H_2O$	0.125 g/L
$MnSO_4 \cdot H_2O$	1.25 g/L
$CoCl_2 \cdot 6H_2O$	0.875 g/L
H_3BO_3	0.06 g/L

$Na_2MoO_4 \cdot 2H_2O$	0.25 g/L
$FeSO_4 \cdot 7H_2O$	5.5 g/L

Solution MXWT-E (prepare fresh, 5 mL needed)
(autoclaved)
Glucose 50 g/L (0.25 g in 5 mL)
This concentration will result in 5.0 g/L in final solution

2) FOR BIOREACTOR A

Basic Medium for 1.50 liter volume:

Solution MXWT-A (prepare fresh, 1200 mL needed)
(autoclaved)

KH_2PO_4	1.80 g/L (2.16 g/1200 mL)
$K_2HPO_4 \cdot 3H_2O$	3.175 g/L (3.81 g/1200 mL)
K_2SO_4	2.5 g/L (3.00 g/1200 mL)
NH_4Cl	4.38 g/L (5.25 g/1200 mL)
$Na_2(EDTA) \cdot 2H_2O$	25.0 mg/L (30 mg/1200 mL)

Adjust to pH 7.0 with 30% (w/v) NaOH

Solution MXWT-B (solution may be stored on counter, 75 mL needed)
(autoclaved)
$MgSO_4 \cdot 7H_2O$ 9.0 g/L

Solution MXWT-C (solution may be stored in refrigerator, 75 mL needed)
(filtered)
thiamine·HCl 0.40 g/L

Solution MXWT-D (solution may be stored on counter, 1.5 mL needed)
(filtered)

Citric acid	50 g/L
$ZnSO_4 \cdot 7H_2O$	0.25 g/L
$CuCl_2 \cdot 2H_2O$	0.125 g/L
$MnSO_4 \cdot H_2O$	1.25 g/L
$CoCl_2 \cdot 6H_2O$	0.875 g/L
H_3BO_3	0.06 g/L
$Na_2MoO_4 \cdot 2H_2O$	0.25 g/L
$FeSO_4 \cdot 7H_2O$	5.5 g/L

Solution MXWT-E (prepare fresh, 150 mL needed)
(autoclaved)

Glucose	120 g/L (18 g in 150 mL)

This concentration will result in 12.0 g/L in final solution

3) FOR BIOREACTOR B

Only change is solution MXWT-A which contains

Solution MXWT-A (prepare fresh, 1200 mL needed)
(autoclaved)

KH_2PO_4	1.80 g/L (2.16 g/960 mL)
$K_2HPO_4 \cdot 3H_2O$	3.175 g/L (3.81 g/960 mL)
K_2SO_4	2.5 g/L (3.00 g/960 mL)
NH_4Cl	1.56 g/L (1.88 g/960 mL)
$Na_2(EDTA) \cdot 2H_2O$	25.0 mg/L (30 mg/960 mL)

Adjust to pH 7.0 with 30% (w/v) NaOH

Volume needed for the final medium (per liter):

MXWT-A	1200 mL
MXWT-B	75 mL
MXWT-C	75 mL
MXWT-D	1.5 mL
MXWT-E	150 mL
Total	1501 mL

Composition of Final Medium

	Bioreactor A	**Bioreactor B**
Component	**Concentration**	**Concentration**
glucose	12.0 g/L	12.0 g/L
NH_4Cl	**3.50 g/L**	**1.25 g/L**
KH_2PO_4	1.44 g/L	1.44 g/L
$K_2HPO_4 \cdot 3H_2O$	2.51 g/L	2.51 g/L
K_2SO_4	2.00 g/L	2.00 g/L
$Na_2(EDTA) \cdot 2H_2O$	20.0 mg/L	20.0 mg/L
$MgSO_4 \cdot 7H_2O$	0.45 g/L	0.45 g/L
$ZnSO_4 \cdot 7H_2O$	0.25 mg/L	0.25 mg/L
$CuCl_2 \cdot 2H_2O$	0.125 mg/L	0.125 mg/L
$MnSO_4 \cdot H_2O$	1.25 mg/L	1.25 mg/L
$CoCl_2 \cdot 6H_2O$	0.875 mg/L	0.875 mg/L
H_3BO_3	0.06 mg/L	0.06 mg/L
$Na_2MoO_4 \cdot 2H_2O$	0.25 mg/L	0.25 mg/L
$FeSO_4 \cdot 7H_2O$	5.50 mg/L	5.50 mg/L
citric acid	50 mg/L	50 mg/L
thiamine·HCl	20 mg/L	20 mg/L