1011c: DESULFOTHERMUS MJ MEDIUM (H2/CO2)

* This recipe might contain strain-specific modifications for *Sulfurovum aggregans* DSM 27205

Final pH: 6.0 - 6.5

NaCl	30.00	g
K_2HPO_4	0.14	g
$CaCl_2$ x 2 H_2O	0.14	g
$MgSO_4$ x 7 H_2O	3.40	g
$MgCl_2$ x 6 H_2O	4.18	g
KCl	0.33	g
NH_4Cl	0.25	g
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O	0.01	g
Trace element solution	10.00	ml
Na_2CO_3	1.50	g
~~Na-pyruvate~~	~~0.50~~	~~g~~
~~Na-lactate~~	~~0.50~~	~~g~~
~~Yeast extract~~	~~0.10~~	~~g~~
$Na_2S_2O_3$ x 5 H_2O	1.50	g
Vitamin solution	10.00	ml
Distilled water	1000.00	ml
~~Sodium dithionite~~	~~20.00~~	~~mg/l~~
$NaNO_3$, might need strain adjustment	1.00	g/l
$NaNO_3$*	1.00	g/l
sulfur*	10.00	g/l

Dissolve ingredients (except carbonate, pyruvate, lactate, yeast extract, thiosulfate and vitamins), then sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials up to a volume of 20% and autoclave. Add pyruvate, lactate, yeast extract, thiosulfate and vitamins to the autoclaved medium from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Solutions of vitamins and thiosulfate are sterilized by filtration. Adjust pH of the complete medium to 6.7. Prior to inoculation reduce medium with 10 - 20 mg/l sodium dithionite, added from a 5% (w/v) solution freshly prepared under N_2 and filter sterilized. After inoculation pressurize vessels to 2 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.

Trace element solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g

$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na-tungstate x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Vitamin solution (from medium 141)

Biotin	2.0	mg
Folic acid	2.0	mg
Pyridoxine hydrochloride	10.0	mg
Thiamine-HCl	5.0	mg
Riboflavin	5.0	mg
Nicotinic acid	5.0	mg
D-Ca-pantothenate	5.0	mg
Vitamin B_{12}	0.1	mg
p-aminobenzoic acid	5.0	mg
Lipoic acid	5.0	mg
Distilled water	1000.0	ml

Supplement medium with 1.00 g/l $NaNO_3$ and omit pyruvate, lactate, yeast extract and dithionite. Upon autoclaving add 10.00 g/l sterile sulfur powder (sterilized by steaming for 3 hours on each of 3 successive days) and adjust pH to 6.0 - 6.5. After inoculation do not pressurize with 80% H_2 and 20% CO_2 gas mixture and add sterile air in an amount that is equivalent to a volume of 10% of the headspace. *