

01/08/18

Methods (without buffer)

Culture and media

The mineral stock solution contained (per liter) the following: 11.7 g NaCl; 2.37g NH₄Cl; 0.65 g CaCl₂·2H₂O; 0.25g MgCl₂·6H₂O; 0.31 g MnCl₂·4H₂O; 0.2 g KH₂PO₄; 0.12g ZnCl₂·H₂O; and 0.048 g CoCl₂·6H₂O.

The vitamin solution contained (per liter) the following: 20 mg pyridoxine; 10 mg each of thiamine, riboflavin, calcium pantothenate, thioctic acid, para aminobenzoic acid, nicotinic acid, and vitamin B12; and 4 mg each of d-biotin, folic acid, and 2-mercaptoethanesulfonic acid.

The media (per liter) were supplemented with 100 ml mineral stock solution, 5 ml vitamin solution, 1.25 g yeast extract, 1.6 g sodium bicarbonate, 0.5 g L-cysteine·HCl, and 10 mM 2-bromoethanesulfonic acid (BES). 5 mM resazurin were added as a buffer and an oxygen indicator.

For 2 L (20 mM acetate, 100 mM ethanol)

-- 200 ml 10X mineral stock solution + 200 ml 10X BES solution (10 mM)

+ 14.6 ml 20X potassium phosphate solution + 2.5 g yeast extract

+ 20 ml 100X resazurin (5 μM) + ml MQ water

-- boiling & bubbling (N₂), oxygen removal

-- 10 ml vitamin solution + 11.6 ml ethanol + 20 ml sodium acetate

+ 20 ml 100X Na₂CO₃ (1.6 g/L) + 20 ml 100X L-cysteine·HCl (0.5 g/L)

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The vitamin solution contained (per liter) the following: 20 mg pyridoxine; 10 mg each of thiamine, riboflavin, calcium pantothenate, thioctic acid, para aminobenzoic acid, nicotinic acid, and vitamin B12; and 4 mg each of d-biotin, folic acid, and 2-mercaptoethanesulfonic acid.

The media (per liter) were supplemented with 100 ml mineral stock solution, 5 ml vitamin solution, 1.25 g yeast extract, 1.6 g sodium bicarbonate, 0.5 g L-cysteine·HCl, and 10 mM 2-bromoethanesulfonic acid (BES). 50 mM potassium phosphate at pH 7.0 and 5 mM resazurin were added as a buffer and an oxygen indicator.

Batch tests (150 ml) (50 mM formic acid, 50, 100, 150 mM ethanol)

- 15 ml 10X mineral stock solution + 15 ml 10X BES solution (10 mM)
 - + 7.5 ml 20X potassium phosphate solution (buffer, 50 mM) + 0.1875 g yeast extract
 - + 1.5 ml 100X resazurin (5 μM) + 4.5 ml 1N NaOH
 - + (102.03, 101.59, 101.16 ml – biomass) MQ water

- boiling & bubbling (CO₂/N₂), oxygen removal

- 0.75 ml vitamin solution + 0.44, 0.87, 1.31 ml ethanol + 0.28 ml formic acid
 - + 1.5 ml 100X Na₂CO₃ (1.6 g/L) + 1.5 ml 100X L-cysteine·HCl (0.5 g/L) + biomass

- pH check and adjustment to 7.0 (1 N NaOH and HCl, pH test paper)

- Control: 1. No ethanol 2. No biomass

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Batch tests (200 ml) (50 mM formic acid, 50, 100, 150 mM ethanol)

- 20 ml 10X mineral stock solution + 0.25 g yeast extract
- + 2 ml 100X resazurin (5 μ M) + 20 ml 10X BES solution (10 mM)
- + 20 ml 10X KH_2PO_4 solution (buffer, 0.1 M)
- + (129.04, 128.46, 127.87 ml – biomass) MQ water

Ps: may need NaOH or Na_2CO_3 to balance the effect of formic acid

-- boiling & bubbling (CO_2/N_2), oxygen removal

- 1 ml vitamin solution + 0.58, 1.16, 1.75 ml ethanol + 0.38 ml formic acid
- + 2 ml 100X L-cysteine·HCl (0.5 g/L) + biomass + 5 ml Na_2CO_3 (4 g/L)

-- pH check and adjustment to 7.0 (1 N NaOH and HCl, pH test paper)

Batch tests (200 ml) (50 mM formic acid, 150 mM ethanol)

- 20 ml mineral stock solution + 0.25 g yeast extract + 2 ml 100X resazurin (5 μ M)
- + 20 ml 10X MES solution (5 g/L) + (134.87 ml – biomass) MQ water

-- boiling & bubbling (N_2), oxygen removal

- 1 ml vitamin solution + 1.75 ml ethanol + 0.38 ml formic acid
- + 20 ml 10X NaHCO_3 solution (0.08 M) + 0.1 g L-cysteine·HCl (0.5 g/L) + biomass

-- pH adjustment to 7.0 (1 N NaOH and HCl, pH test paper)

Methods

Culture and media

The mineral stock solution contained (per liter) the following: 11.7 g each NaCl and $(\text{NH}_4)_2\text{SO}_4$; 11.25 g KH_2PO_4 ; 0.65 g each $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.26 g each $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and $\text{ZnSO}_4 \cdot 2\text{H}_2\text{O}$; and 0.048 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$.

The vitamin solution contained (per liter) the following: 20 mg pyridoxine; 10 mg each of thiamine, riboflavin, calcium pantothenate, thioctic acid, para aminobenzoic acid, nicotinic acid, and vitamin B12; and 4 mg each of d-biotin, folic acid, and 2-mercaptoethanesulfonic acid.

The media (per liter) were supplemented with 100 ml mineral stock solution, 5 ml vitamin solution, 1.25 g yeast extract, 5 g morpholinoethanesulfonic acid, and 6.72 g sodium bicarbonate. 5 mM resazurin was added as an oxygen indicator.

Produces (for 500 ml):

-- 50 ml mineral stock solution + 0.625 g yeast extract + 5 ml 100X resazurin solution
+ sodium acetate + 50 ml 10X MES solution
+ (342.5 ml – ethanol – biomass) MQ water

-- boiling & oxygen removal

-- 2.5 ml vitamin solution + 50 ml 10X NaHCO_3 solution + ethanol + 0.25g L-cysteine·HCl + biomass

-- pH adjustment (1 N NaOH and HCl)

