

SUPPLEMENTARY INFORMATION

S.1 Solution preparation for MES operation

P7 culture medium

The P7 medium was composed of $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ (0.2 g), KH_2PO_4 (0.1 g), KCl (0.1 g), NaCl (0.8 g), NH_4Cl (1 g), $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ (0.02 g), Yeast Extract (1 g), NaHCO_3 (1 g). All medium compounds were dissolved in a total volume of 1 L of deionized water. A volume of 0.5 mL of resazurin (0.1% w/w) was then added as an indicator of redox state. The solution was distributed into 100 mL serological bottles, and these bottles were then heated for 5 minutes in a microwave. Each bottle was then flushed with N_2/CO_2 (80:20 vol/vol) for 5 minutes. Vitamin stock solution (1 mL; for composition see below), cysteine 30 g/L (1 mL), and 2-(N-morpholino) ethanesulfonic acid (MES) buffer 200 g/L (5 mL), were also added into each bottle.

Clostridium kluyveri culture medium

Clostridium kluyveri culture medium was composed of $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ (0.2 g), KH_2PO_4 (0.1 g), KCl (0.1 g), NaCl (0.8 g), NH_4Cl (1 g), $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ (0.02 g), Yeast Extract (1 g), NaHCO_3 (1 g) dissolved in a total volume of 1 L of deionized water. A volume of 0.5 mL of resazurin (0.1% w/w) was added as an indicator of redox state. The solution was distributed into 100 mL serological bottles, and these bottles were then sterilized by heating for 5-10 minutes in a microwave. Each bottle was then flushed with N_2/CO_2 (80:20 vol/vol) for 5 minutes to establish anaerobic conditions. Vitamin stock solution (1 mL; for composition see below), cysteine 30 g/L (1 mL), MES 200 g/L (10 mL), sodium acetate

300 g/L (1 mL), absolute ethanol (0.6 mL) and NaHCO₃ 50 g/L (2 mL) were added into each bottle.

Vitamin solution

The vitamin solution was prepared by introducing in 1L of distilled water biotin (2 mg), folic acid (2 mg), pyridoxine-HCl (10 mg), thiamine-HCl 2 H₂O (5 mg), riboflavin (5 mg) nicotinic acid (5 mg) and D-ca-pantothenate (5 mg).

Minerals solution

The mineral aqueous solution contains NH₄Cl (100 g L⁻¹), NaCl (80 g L⁻¹), KCl (10 g L⁻¹), KH₂PO₄ (10 g L⁻¹), MgSO₄.7H₂O (20 g L⁻¹), CaCl₂.H₂O (4 g L⁻¹).

Trace elements solution

Trace elements solution consisted of the following chemicals: Na-EDTA (0,5 g L⁻¹), FeCl₂.4H₂O (2 g L⁻¹), H₃BO₃ (0,05 g L⁻¹), ZnCl₂ (0,05 g L⁻¹), CuCl₂.2H₂O (0,04 g L⁻¹), MnCl₂.4H₂O (0,5 g L⁻¹), (NH₄)₆Mo₇O₂₄.4H₂O (0,05 g L⁻¹), AlCl₃.7H₂O (0,054 g L⁻¹), CoCl₂.6H₂O (0,15 g L⁻¹), NiCl₂.6H₂O (0,1 g L⁻¹), MgCl₂.6H₂O (1 g L⁻¹), Na₂Se₃O₃ (0,05 g L⁻¹).

S.2 Droplet Digital PCR (ddPCR) method

The PCR mixture consisted of 12.5 uL Bio-Rad 2x QX200 ddPCR EvaGreen^R Supermix (SM) (BioRad Laboratories Ltd, Mississauga, ON), primers at a final

concentration of 0.1 μM , 0.2 mg/mL BSA and genomic DNA input concentrations 1 ng DNA per 25 μL ddPCR reaction.

Sample mixtures were gently vortexed avoiding the formation of bubbles, centrifuged for 20 sec, then kept on ice until droplet generation. Samples were packaged into droplets by adding 20 μL of the PCR mixture to each sample well of the single-use DG8 cartridge followed by addition of 70 μL of droplet generation oil for EvaGreen^R to each of the corresponding oil wells. The cartridge was then placed into the QX200 droplet generator for droplet production. Forty microliters of generated droplets were transferred from the cartridge to a semi-skirted ddPCR 96-well plate (Bio-Rad). Samples were successively prepared in cartridges in groups of eight, transferred to the PCR plate and subsequently heat sealed with a pierceable foil seal. The plate was transferred to a thermal cycler, and reactions were run under the following standard cycling conditions: 95°C for 5 min followed by 40 cycles of 95°C for 30 sec, 59°C for 60 sec, 72°C for 30 sec. The cooling step was performed at 4°C for 5 min followed by a final extension at 90°C for 5 min then a 4°C hold; ramp rate = 50% (2°C/sec). Upon completion of the PCR phase, plates were loaded onto the QX200 Droplet Digital reader, which automatically reads the droplets from each well of the plate. Data analysis was performed using QuantaSoftTM software (Bio-Rad). Negative droplets, lacking target and/or reference gene DNA, as well as positive droplets containing at least one copy of the target were counted. The concentration of target DNA was determined using Poisson distribution analysis.

S.3 Dissolved CO₂ analysis method

An equimolar aqueous solution of potassium carbonate and sodium carbonate was prepared at 10 g/L. Different solutions (5 g/L; 2.5 g/L; 1.25 g/L) were prepared by successive dilution of this stock solution.

For each solution prepared, 0.4 mL was introduced into a 10 mL vial and sealed. A volume of 0.5 mL of 3 M HCl solution was added using a syringe. The carbon dioxide evolved after this acidification of the solution was analyzed using a gas chromatograph. A curve between the chromatographic peak of carbon dioxide and the concentration of carbonate was then plotted. The resulting linear correlation is used to determine the concentration of CO₂ dissolved in the MES cell samples.