Electronic Supplementary Information to: Production of isobutyric acid from methanol by *Clostridium luticellarii*

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A.1. Material and Methods

Continuous mixed-culture reactor

A 1.2 L Continuous Stirred Tank Reactor (CSTR) with a working volume of 0.9 L (Figure S1.) was operated for 38 days and served as a source of inoculum for isolation. The reactor contained a mixed-culture dominated by organisms from the genera *Eubacterium* and *Clostridium*, able to stably produce isobutyric acid from methanol (Huang et al., 2020). Carbon and nutrients were fed into the reactor from two independent 5.0 L vessels stored at 4°C and through two different lines to avoid feed contamination. One vessel contained carbon and energy source, i.e. methanol 6.4 $g.L^{-1}$ (200 mM), potassium acetic acid 9.8 $g.L^{-1}$ (100 mM) and sodium bicarbonate (90 mM). The other contained nitrogen, phosphorus and other micro-nutrient sources which exact composition can be found in Table S1. The reactor pH was controlled at 6.30 using a DULCOMETER® D1Cb/D1Cc pH controller (Prominent, Germany) by automatic addition of 1.5M HCl. The reactor was fed at a rate of 0.35 ± 0.04 $L \cdot d^{-1}$ using a peristaltic pump (Watson Marlow IP31, UK) and set in a temperature-controlled room to maintain the fermentation temperature at 34 ± 1 °C. Gas production was measured with a calibrated gas counter based on volume displacement. Liquid samples from the effluent and gaseous outflows were taken 2-3 times a week.

A.2. Figures and Tables

Table S1. Medium composition of the CSTR fermenting methanol and acetic acid. The compositions of the vitamin and trace element stock solutions are indicated in Table S2 to Table S5.

	Compound	Amount per L	Unit
Carbon – vessel			
	$NH_4H_2PO_4$	3.600	g
	$MgCl_2.6H_2O$	0.325	g
	$MgSO_4.7H_2O$	0.197	g
	$CaCl_2.2H_2O$	0.200	g
	KCl	0.149	g
Carbon + vessel			
	Methanol	8.091	mL
	Potassium Acetate	9.815	g
	$NaHCO_3$	7.561	g
	Trace element solution I	10.00	mL
	Trace element solution II	10.00	mL
	Vitamin solution I	10.00	mL
	Vitamin solution II	3.33	mL

Table S2. Composition of the trace element solution I, 1000x concentrated.

Compound	Amount per L	Unit
$ZnSO_4 \cdot 7H_2O$	1.00	g
$MnCl_2 \cdot 4H_2O$	0.30	g
H_3BO_3	3.00	g
$CoCl_2 \cdot 6H_2O$	2.00	g
$CuCl_2 \cdot 2H_2O$	0.10	g
$NiCl_2 \cdot 6H_2O$	0.20	g
$Na2MoO_4 \cdot 2H_2O$	0.30	g
Na_2SeO_3	0.20	g

Table S3. Composition of the trace element solution II, 1000x concentrated.

Compound	Amount per L	Unit
$FeCl_2 \cdot 4H_2O$	15.00	g
HCl (4.0 M)	10.00	mL

Table S4. Composition of the vitamin solution I, 1000x concentrated.

Compound	Amount per L	Unit
Biotin (B7)	0.106	g
Folic acid (B9)	0.005	g
Pyridoxal-HCl (B6)	0.003	g
Lipoic acid	0.015	g
Riboflavin (B2)	0.013	g
Thiamine-HCl (B1)	0.266	g
Ca-D-pantothenate (B5)	0.413	g

4(P)-aminobenzoic acid (B10)	0.013	g
Nicotinic acid (B3)	0.013	g

Table S5. Composition of the vitamin solution II, 3000x concentrated.

Compound	**Amount per L**	**Unit**
Cyanocobalamin (B12)	0.039	g

Table S6. Composition of the filter-sterilised vitamin and trace element solution.

Compound	**Amount per L**	**Unit**
Trace element solution I	33.33	mL
Vitamin solution I	33.33	mL
Vitamin solution II	11.11	mL

Table S7. Composition of the filter-sterilised iron II chloride solution.

Compound	**Amount per L**	**Unit**
Trace element solution II	33.33	mL

Table S8. Composition of the substrate filter-sterilised stock solutions.

	Compound	Amount per L	Unit
Methanol solution			
	Methanol	405	mL
Ethanol solution			
	Ethanol	584	mL
Acetic acid solution			
	Potassium acetate	490.76	g
Lactic acid solution			
	Sodium DL-lactate solution 60%	933.80	g
Propionic acid solution			
	Sodium propionate	480.35	g
Butyric acid solution			
	Sodium butyrate	124.96	g
Valeric acid solution			
	Sodium valerate	310.28	g
Caproic acid solution			
	Sodium hexanoate	345.35	g
Glucose solution			
	Glucose dihydrate	225.20	g
Glycerol solution			
	Glycerol 87%	198.88	g

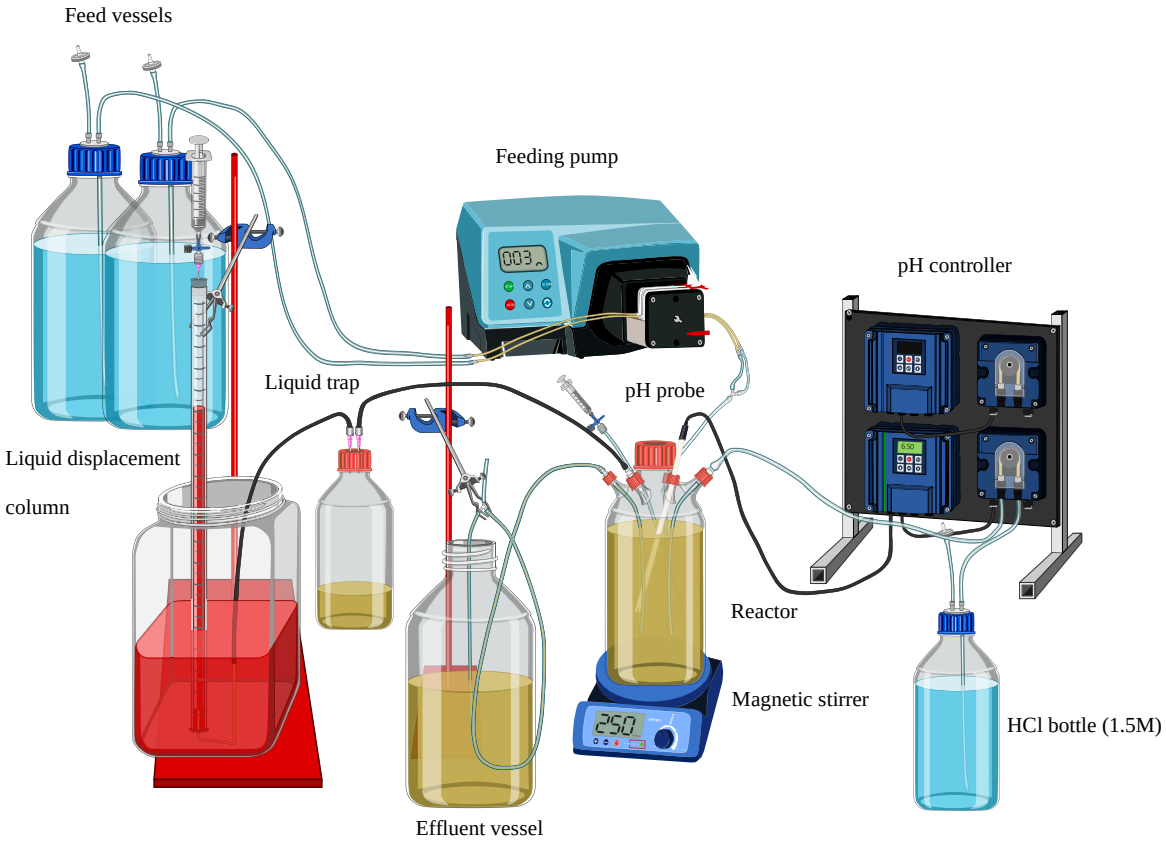

Figure S1. Schematic diagram of the CSTR fermenting methanol and acetic acid.

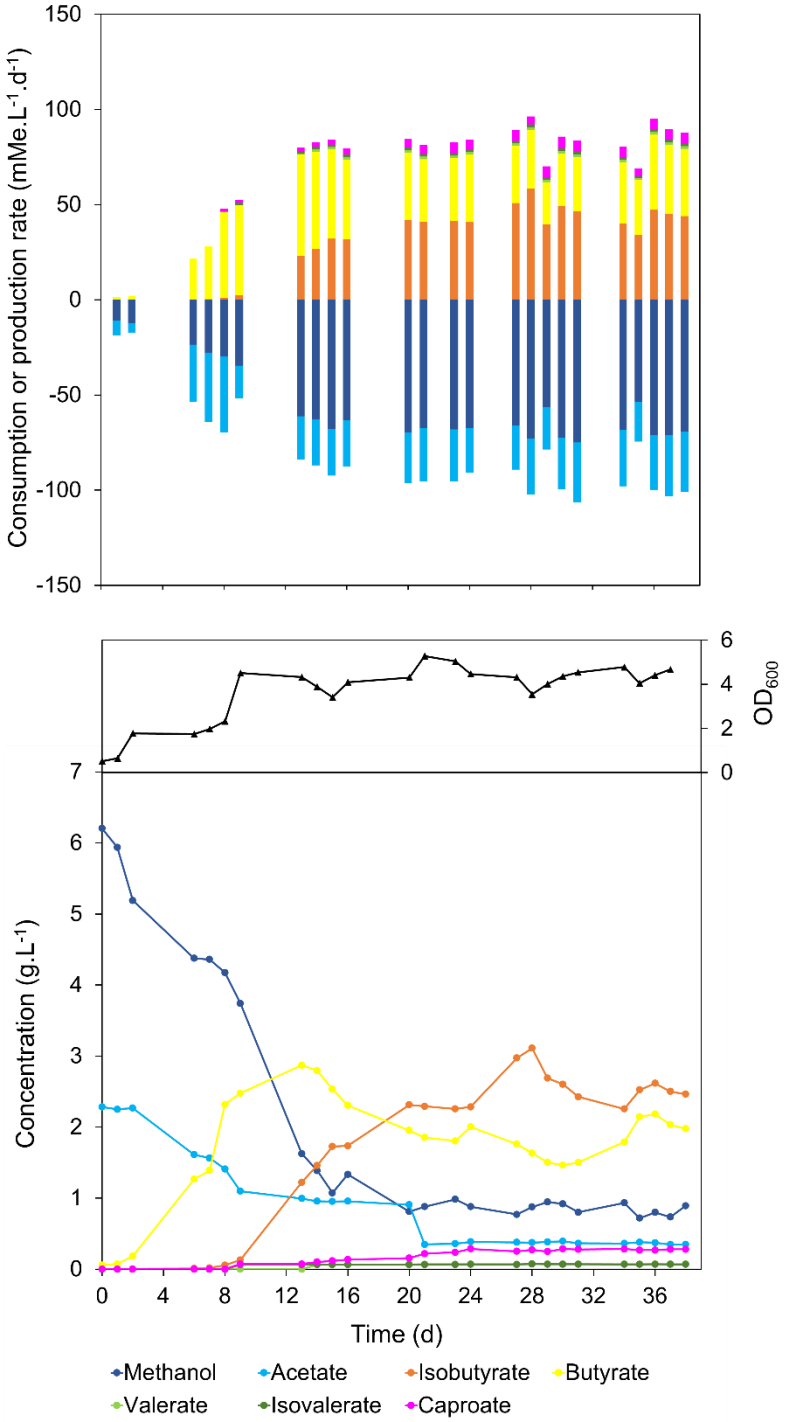

Figure S2. Product formation and substrate consumption rates (Panel A) over the stable operational period of the CSTR reactor fermenting methanol and acetic acid. Optical density and metabolites concentration profile throughout the stable operational period (Panel B).

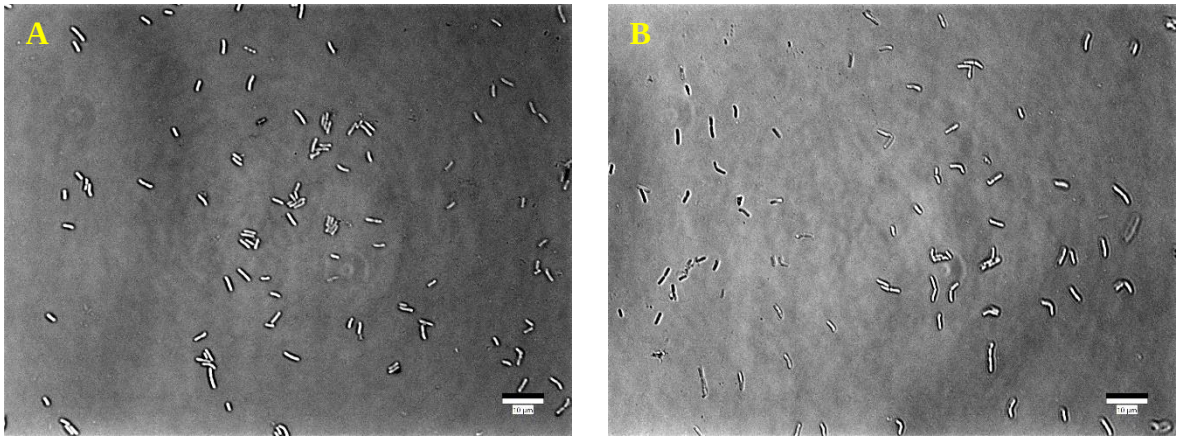

Figure S3. Phase-contrast micrograph showing the cell morphology of isolate R1 (Panel A) and M8 (Panel B). Bars: 10µm (A); 10µm (B).

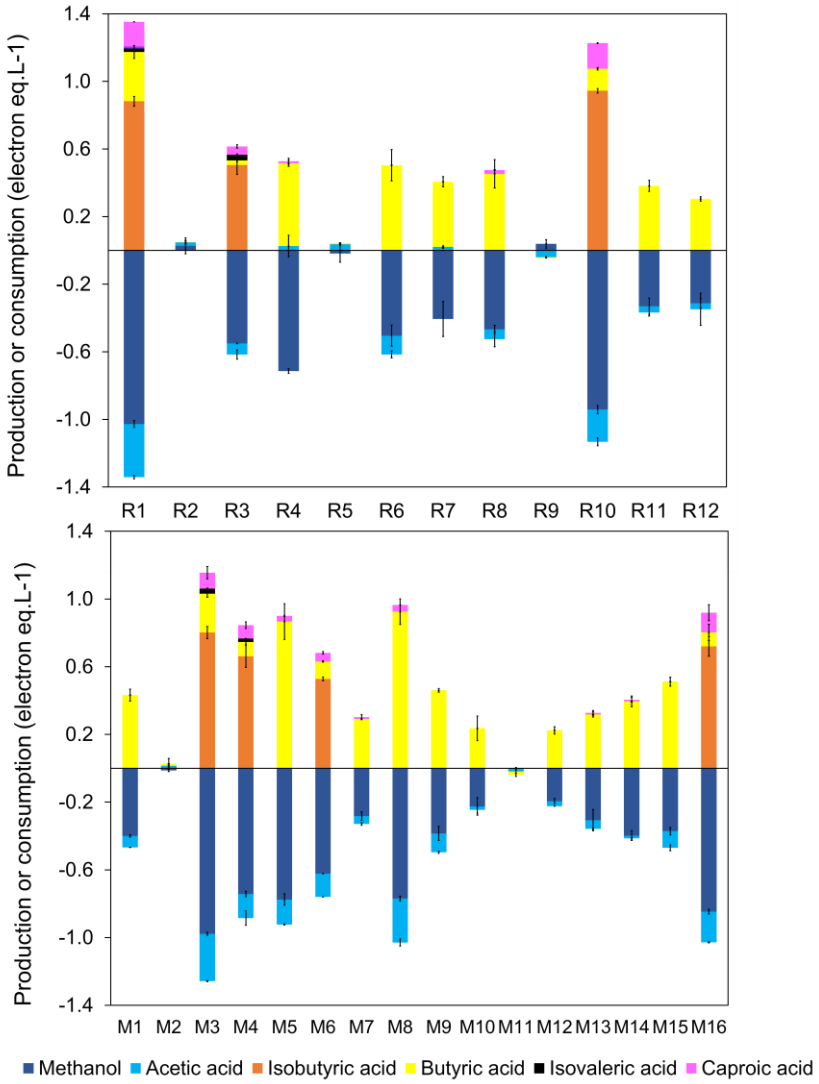

Figure S4. Electron balance of batch incubations of methylotrophic isolates obtained by plating on RCM (Panel A) and methanol synthetic medium (Panel B). Bacterial isolate codes: Letters

R and M indicate the medium they were isolated from with R=Reinforced Clostridial medium and M= Methanol synthetic medium. Numbers refer to specific isolate.

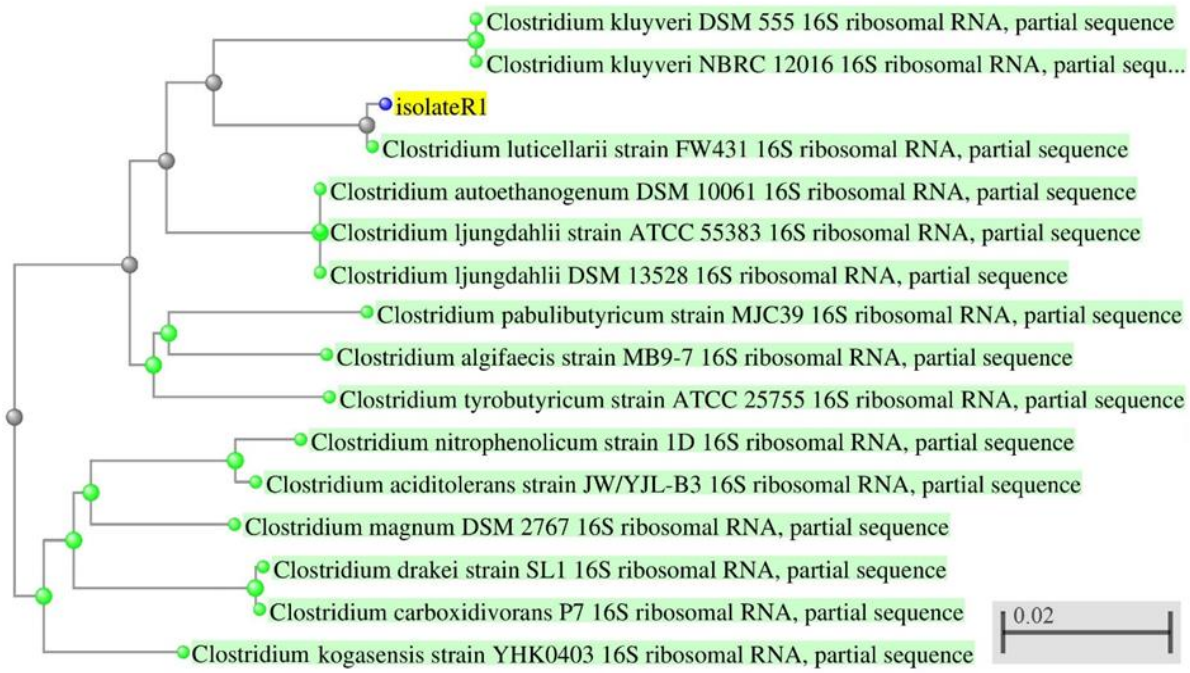

Figure S5. Phylogenetic tree of isolate R1 based on 16s rRNA sequences from the NCBI database. The tree was constructed using the neighbour-joining method. Bar 0.02 indicates substitutions per nucleotide position.

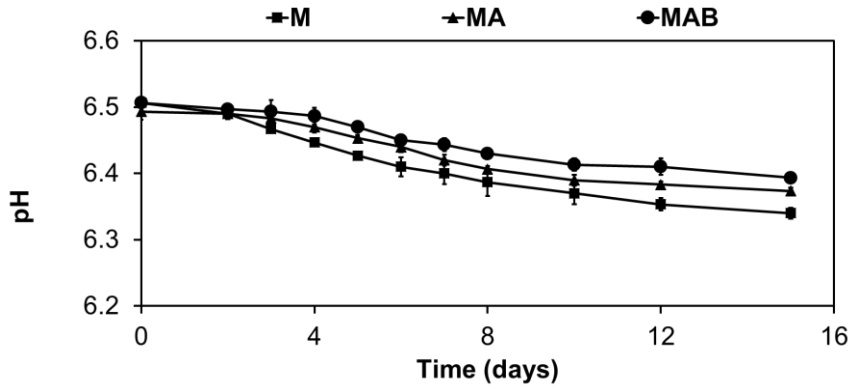

Figure S6. Evolution of pH throughout the batch growth of *C. luticellarii* DSM 29923 on methanol (M),and methanol and acetic acid with (MAB) and without (MA) butyric acid supplementation.