Electronic Supplementary Information

Metabolic analysis of butanol production from acetate in *Clostridium saccharoperbutylacetonicum* N1-4 using ^{13}C tracer experiments

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Table S1. Additional parameters of batch cultures in TY medium containing exogenously added acetate

Initial acetate (g/L)	Glucose consumption[a] (g/L)	Maximum glucose consumption rate (g/L/h)	Acetate/glucose consumption[a,b] (C-mol/C-mol)	Acetone yield[a,c] (C-mol/C-mol)	Ethanol yield[a,d] (C-mol/C-mol)	Ratio of acetone/butanol[a] (g/g)	Maximum specific butanol production[e] rate (g/g/h)
A0	33.6	1.44 (24h)	——	0.150	0.0204	0.384	0.245 (18h)
A2	46.5	2.67 (18h)	0.0363	0.153	0.0436	0.354	0.529 (9h)
A4	46.2	2.54 (18h)	0.0739	0.193	0.0300	0.488	0.540 (9h)
A6	46.2	2.48 (18h)	0.107	0.231	0.0250	0.642	0.331 (18h)

Batch cultures were performed using *C. saccharoperbutylacetonicum* N1-4 at 30ºC, for 72, h in TY medium containing 50 g/L glucose, without pH control (working volume, 300 ml).

[a] The parameters were calculated after 72-h cultivation period. [b] Acetate/glucose consumption (C-mol/C-mol) = Acetate consumption (mM) × 2/glucose consumption (mM) × 6. [c] Acetone production/total substrates consumption (C-mol/C-mol) = Acetone production (mM) × 3/[glucose consumption (mM) × 6 + Acetate consumption (mM) × 2]. [d] Ethanol production / total substrates consumption (C-mol/C-mol) = Ethanol production (mM) × 2/[glucose consumption (mM) × 6 + Acetate consumption (mM) × 2]. [e] Specific butanol production rate (g/g/h) = 2 × (C_2 - C_1)/ (t_2 - t_1)/ (X_2 +

X_1), where C is the butanol concentration (g/L), t is the sampling time (h) and X_1 and X_2 are the dry cell weight at $t_1$and t_2, respectively (g/L).

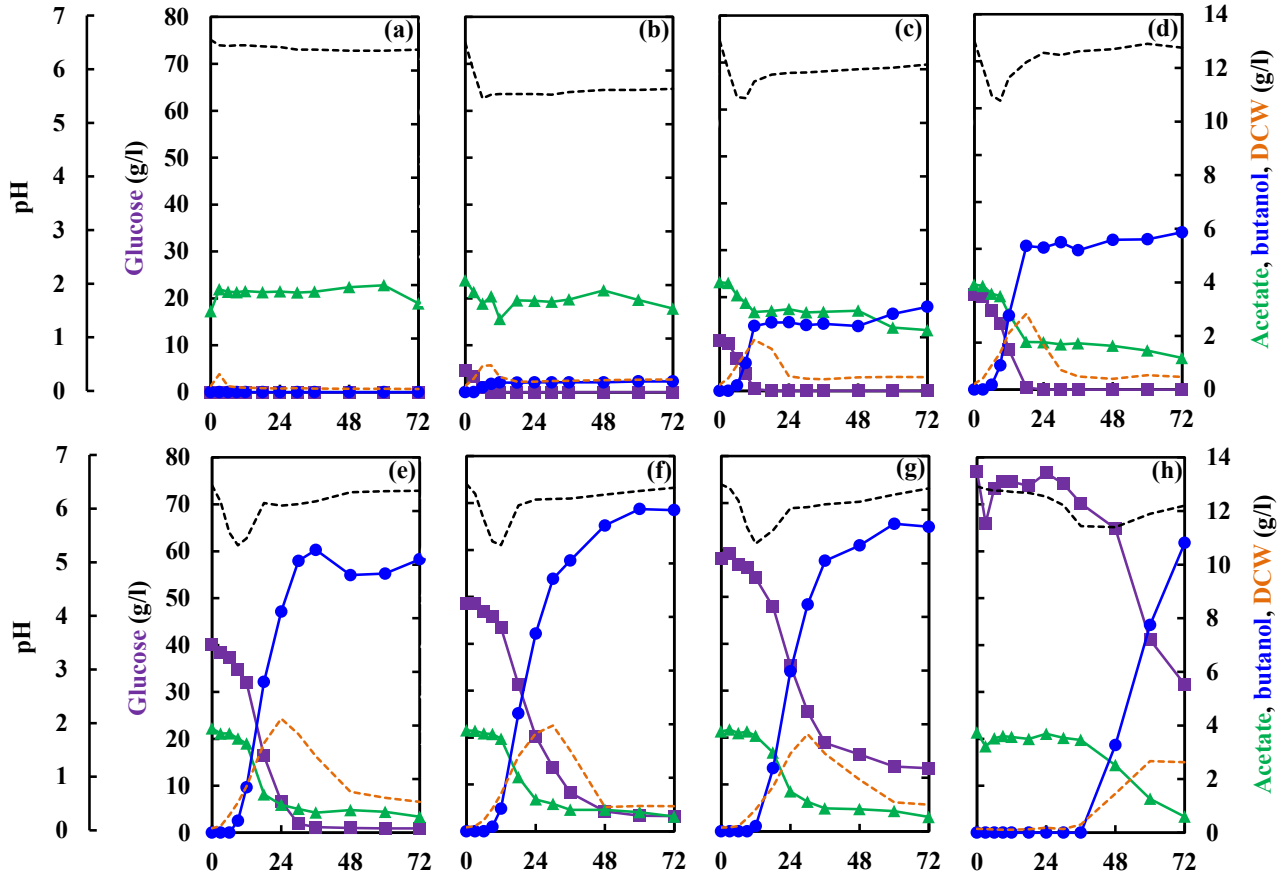

Fig. S1 Time course of batch culture with different concentrations of glucose ranging from 0–80 g/L (working volume, 70 ml). (a) G0; (b) G4; (c) G10; (d) G20; (e) G40; (f) G50; (g) G60; (h) G80. (G: glucose). Symbols: butanol concentration in the broth, blue *circles*; acetate, green *triangles*; glucose, purple *squares*; pH, black *dashed line*; DCW, brown *dashed line*.

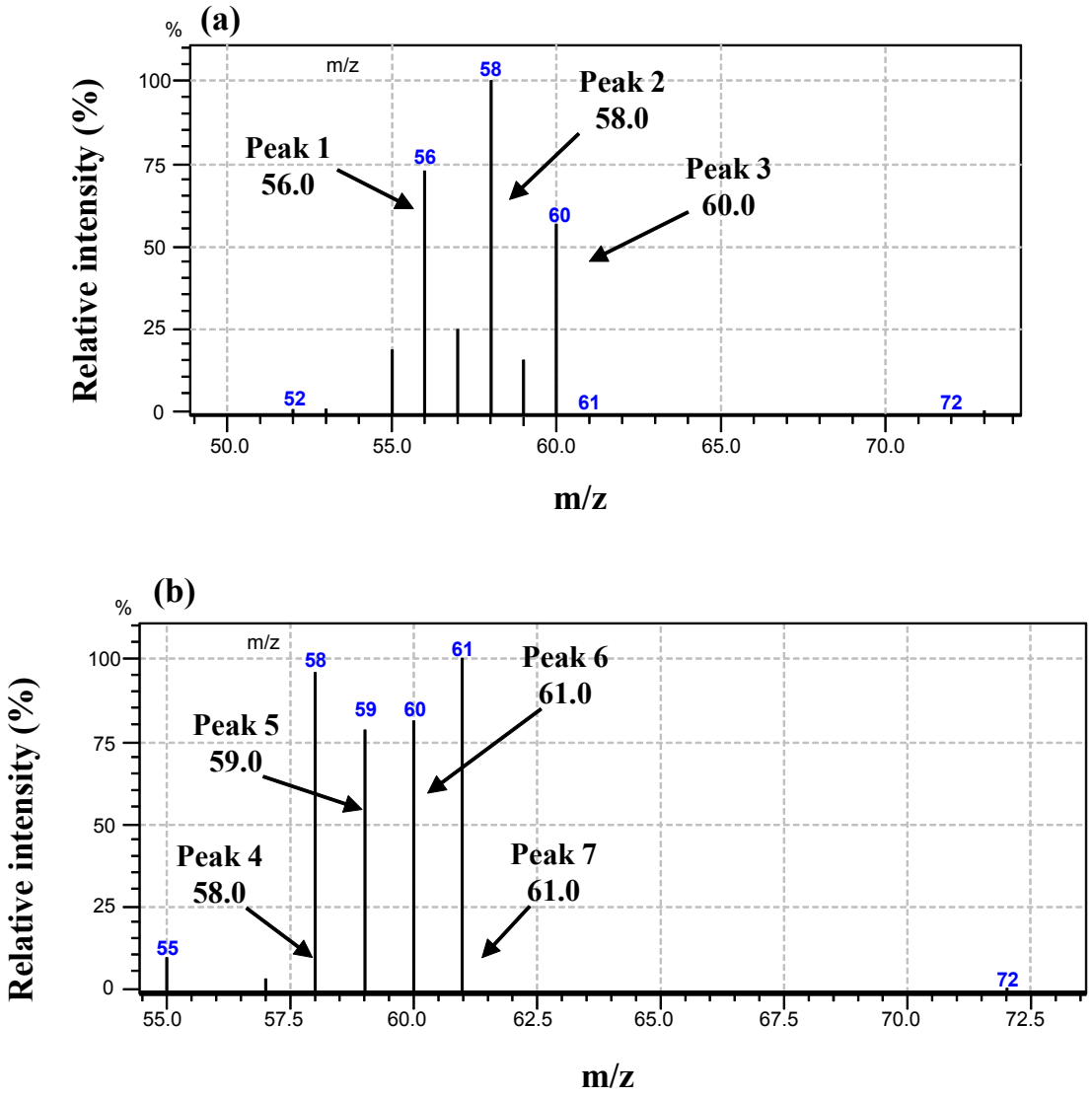

Fig. S2 Mass spectra of butanol and acetone by GC-MS analysis. (a) Mass spectra of butanol in culture broth of the N1-4 strain, cultured with $^{12}C_6$-glucose and [1, 2-$^{13}C_2$] acetate at 9 h. Peak 1, 2 and 3 (indicated by arrows), were derived from three isotopes of butanol: $^{12}C_4$-butanol; $^{13}C_2$-butanol, in which two out of four carbons were replaced by a ^{13}C-atom; and $^{13}C_4$-butanol, respectively. (b) Mass spectra of acetone in culture broth of the N1-4 strain, cultured with $^{12}C_6$-glucose and [1, 2-$^{13}C_2$] acetate at 9 h. Peak 4, 5, 6 and 7, indicated by arrows, were derived from four isotopes of acetone: $^{12}C_3$-acetone; $^{13}C_1$-acetone, in which one out of three carbons was replaced by ^{13}C-atom; $^{13}C_2$-acetone, in which two out of three carbons were replaced by ^{13}C-atom; and $^{13}C_3$-acetone, respectively. X-axis: m/z (mass-to-charge ratio).