

**Supplementary information: Cooperative growth of *Geobacter sulfurreducens* and *Clostridium pasteurianum* with subsequent metabolic shift in glycerol fermentation**

**Roman Moscoviz, Florence de Fouchécour, Gaëlle Santa-Catalina, Nicolas Bernet and Eric Trably\***

\*Corresponding author: *eric.trably@inra.fr*

LBE, INRA, 102 Avenue des étangs, 11100 Narbonne, France

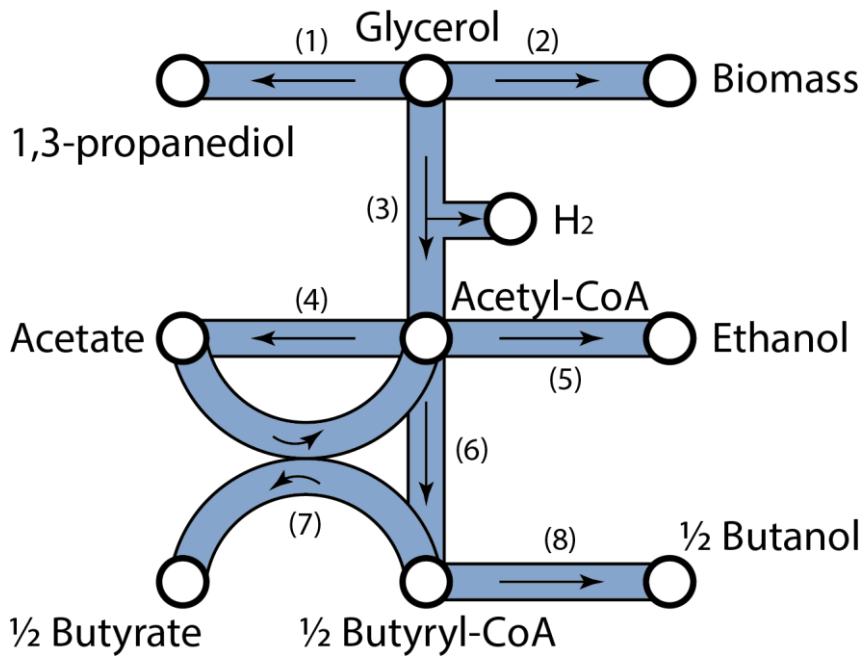


Figure S1: Simplified catabolic pathways of glycerol fermentation by *Clostridium pasteurianum*.

Table S1: Condensed metabolic pathways of glycerol fermentation

Reaction	Legend	Ref
Glycerol + NADH → PDO + H <sub>2</sub> O	(1)	1,2
Glycerol + ¾ NH <sub>3</sub> + 7.5 ATP + 6 H <sub>2</sub> O → ¾ C <sub>4</sub> H <sub>7</sub> O <sub>2</sub> N + NADH	(2)	1
Glycerol + CoA → Acetyl-CoA + CO <sub>2</sub> + H <sub>2</sub> + ATP + H <sub>2</sub> O + 2 NADH	(3)	1,2
Acetyl-CoA → Acetate + ATP + CoA + H <sub>2</sub> O	(4)	1–3
Acetyl-CoA + 2 NADH → Ethanol + CoA	(5)	1–3
2 Acetyl-CoA + 2 NADH → Butyryl-CoA + CoA + H <sub>2</sub> O	(6)	3–5
Butyryl-CoA + Acetate → Butyrate + Acetyl-CoA	(7)	3–5
Butyryl-CoA + 2 NADH → Butanol	(8)	6,7

For more readability, NAD<sup>+</sup>, H<sup>+</sup> and ADP are omitted in the presented equations. C<sub>4</sub>H<sub>7</sub>O<sub>2</sub>N correspond to the mean raw formula of bacterial biomass<sup>1</sup>.

Table S2: Redox and ATP balanced reactions of glycerol metabolism

Global reaction	$\Delta rG^\circ$ (kJ.mol <sub>glycerol</sub> <sup>-1</sup> )
53 Glycerol + 3 NH <sub>3</sub> → 3 C <sub>4</sub> H <sub>7</sub> O <sub>2</sub> N + 15 Acetate + 15 CO <sub>2</sub> + 15 H <sub>2</sub> + 34 PDO + 25 H <sub>2</sub> O	-52.2
38 Glycerol + 3 NH <sub>3</sub> → 3 C <sub>4</sub> H <sub>7</sub> O <sub>2</sub> N + 30 Ethanol + 30 CO <sub>2</sub> + 30 H <sub>2</sub> + 4 PDO + 10 H <sub>2</sub> O	-50.1
48 Glycerol + 3 NH <sub>3</sub> → 3 C <sub>4</sub> H <sub>7</sub> O <sub>2</sub> N + 10 Butyrate + 20 CO <sub>2</sub> + 20 H <sub>2</sub> + 24 PDO + 40 H <sub>2</sub> O	-109.1
38 Glycerol + 3 NH <sub>3</sub> → 3 C <sub>4</sub> H <sub>7</sub> O <sub>2</sub> N + 15 Butanol + 30 CO <sub>2</sub> + 30 H <sub>2</sub> + 4 PDO + 25 H <sub>2</sub> O	-92.3

*Equations were balanced using equations from Table S1. Standard Gibbs free energy of reaction ( $\Delta rG^\circ$ ', for pH 7 and T = 25 °C) were calculated using Gibbs free energy of formation from Kleerebezem and Van Loosdrecht (2010)<sup>8</sup>.*

## Bibliography

1. Zeng, A.-P., Biebl, H., Schlieker, H. & Deckwer, W.-D. Pathway analysis of glycerol fermentation by *Klebsiella pneumoniae*: regulation of reducing equivalent balance and product formation. *Enzyme Microb. Technol.* **15**, 770–779 (1993).
2. Zeng, A.-P. Pathway and kinetic analysis of 1, 3-propanediol production from glycerol fermentation by *Clostridium butyricum*. *Bioprocess Eng.* **14**, 169–175 (1996).
3. Temudo, M. F., Kleerebezem, R. & van Loosdrecht, M. Influence of the pH on (open) mixed culture fermentation of glucose: A chemostat study. *Biotechnol. Bioeng.* **98**, 69–79 (2007).
4. Louis, P. & Flint, H. J. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol. Lett.* **294**, 1–8 (2009).
5. Vital, M., Howe, A. C. & Tiedje, J. M. Revealing the bacterial butyrate synthesis pathways by analyzing (meta) genomic data. *MBio* **5**, e00889–14 (2014).
6. Atsumi, S. *et al.* Metabolic engineering of *Escherichia coli* for 1-butanol production. *Metab. Eng.* **10**, 305–311 (2008).
7. Jin, C., Yao, M., Liu, H., Lee, C. F. & Ji, J. Progress in the production and application of n-butanol as a biofuel. *Renew. Sustain. Energy Rev.* **15**, 4080–4106 (2011).
8. Kleerebezem, R. & Van Loosdrecht, M. C. M. A Generalized Method for Thermodynamic State Analysis of Environmental Systems. *Crit. Rev. Environ. Sci. Technol.* **40**, 1–54 (2010).