Supplementary material

Influence of fumarate on electron transfer between *Geobacter sulfurreducens* and *Clostridium pasteurianum*

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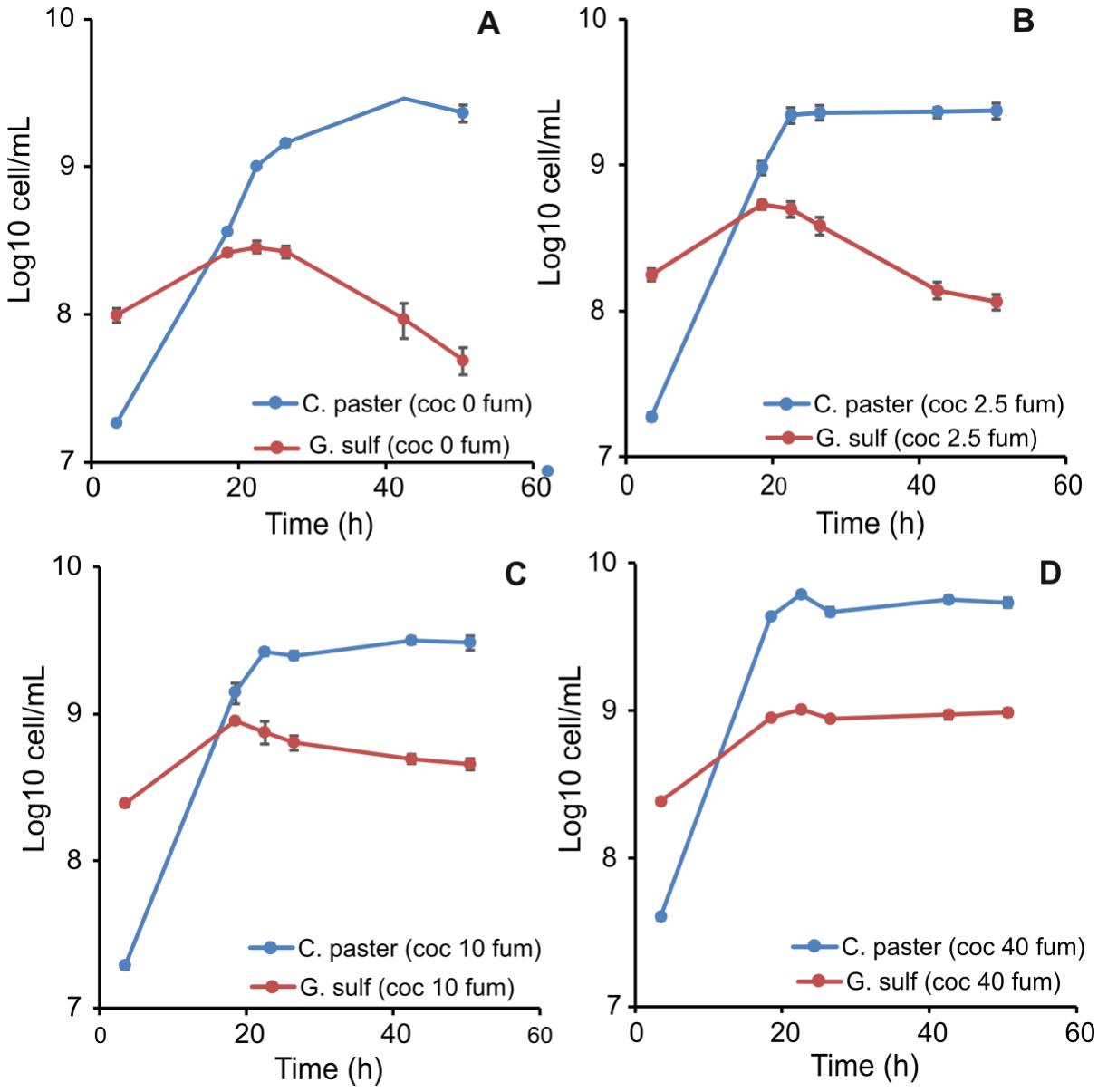

Figure S1. qPCR-based kinetics of *C. pasteurianum* and *G. sulfurreducens* during co-cultures with **A)** 0 mM fumarate, **B)** 2.5 mM fumarate, **C)**10 mM fumarate, and **D)** 40 mM fumarate.

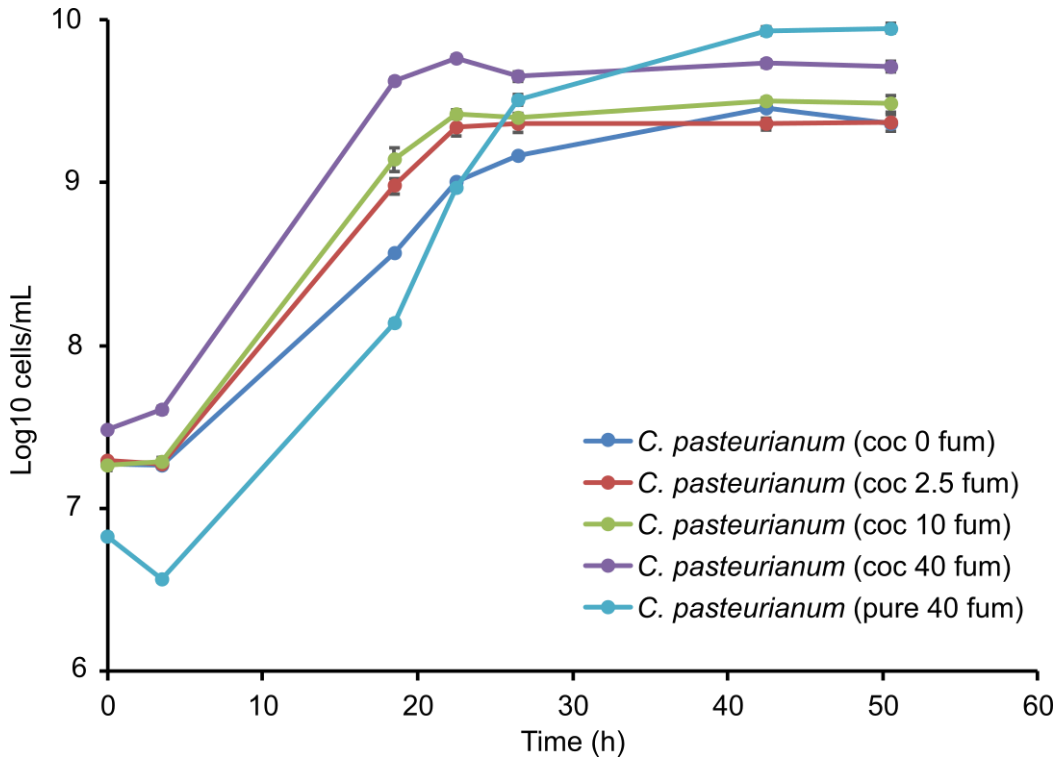

Figure S2. qPCR-based comparison kinetics of *C. pasteurianum* grown in pure cultures and in co-culture with *G. sulfurreducens* at different fumarate concentrations.

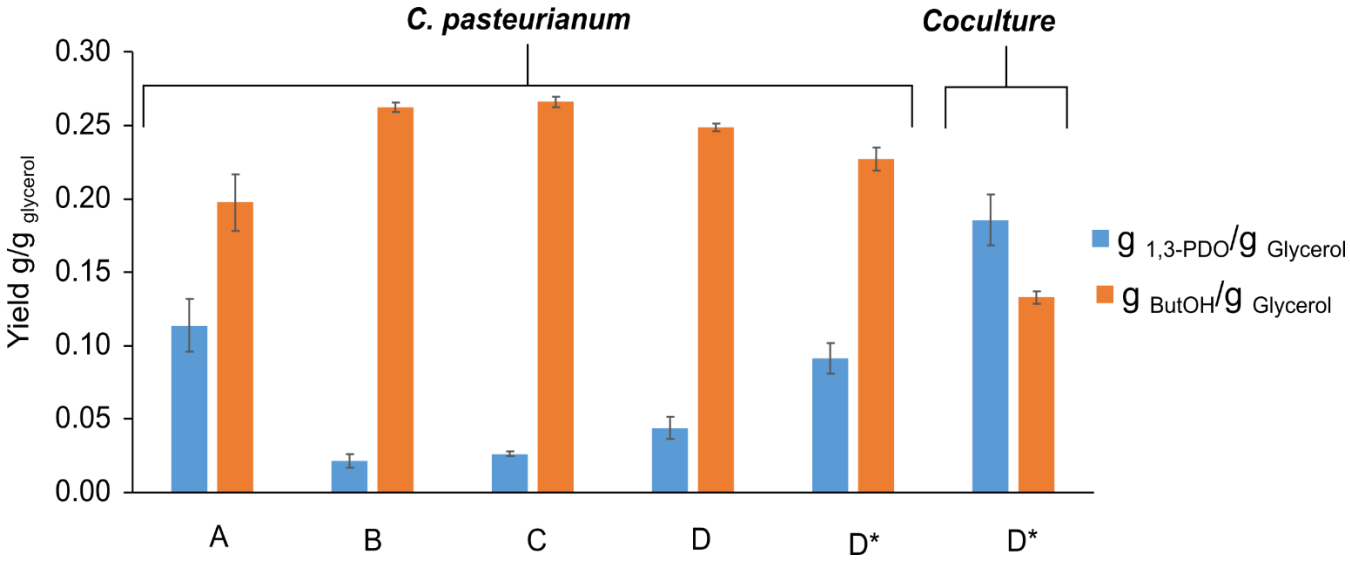

Figure S3. Comparison of 1,3-PDO and butanol yields from glycerol fermentation by *Clostridium pasteurianum* under different conditions. A: only with glycerol, B: plus 20 mM succinate, C: plus 40 mM succinate, D: plus 20 mM acetate/40 mM fumarate. *Cultures refer to the experiments reported in the main manuscript.

Statistical analysis: ANOVA + Tukey's honest significant difference test (HSD), 5% significance

Table S1. Initial (average) and maximum number of *G. sulfurreducens* cells in pure and in co-culture without fumarate addition.

	***G. sulfurreducens* initial**	***G. sulfurreducens* pure**	***G. sulfurreducens* co-culture**
Average	2.01E+08	2.09E+08	2.86E+08
SD	3.45E+07	7.92E+06	2.51E+07

Table S2. ANOVA initial and maximum number of *G. sulfurreducens* cells in pure and in co-culture without fumarate addition.

Groups	***No. samples***	***Sum***	***Average***	***Variance***
***G. sulfurreducens* initial**	3	603614855	201204952	1.189E+15
***G. sulfurreducens* pure**	3	627794252	209264751	6.2759E+13
***G. sulfurreducens* co-culture**	3	858782201	286260734	6.2841E+14

P-value=0.011

Table S3. The difference in average G. sulfurreducens cells between each condition. In red, conditions with significant differences according to **HSD= 6.27E+07**

Groups	*G. sulfurreducens* initial	G. sulfurreducens pure	G. sulfurreducens co-culture
G. sulfurreducens initial		8.06E+06	8.51E+07
G. sulfurreducens pure			7.70E+07
G. sulfurreducens co-culture			

Table S4. Butanol, 1,3-PDO and butyrate yields data used for statistical analysis

Yields mmol*mol glycerol	**C. pasteurianum**	**[0 mM fumarate]**	**[2.5 mM fumarate]**	**[10 mM fumarate]**	**[40 mM fumarate]**
Butanol	277.9 ± 6.8	231.0 ± 0.3	222.1± 3.2	248.0 ± 7.2	167.9 ± 3.8
1-3 PDO	108.8 ± 8.7	174.3 ± 3.8	178.9 ± 3.6	164.2 ± 5.9	228.3 ± 14.5
butyrate	102 ± 25	122 ± 3	122 ± 1	103 ± 0.3	103 ± 6

Table S5. ANOVA for butanol yields

Groups	***No. samples***	***Sum***	***Average***	***Variance***
C. pasteurianum	3	833.7184483	278	45.97601198
[0 mM fumarate]	3	692.8573879	231	0.06288194
[2.5 mM fumarate]	3	666.1541289	222	10.12726127
[10 mM fumarate]	3	744.0631348	248	52.47924902
[40 mM fumarate]	3	503.8216209	168	14.4814537

P-value = 1.75E-09

Table S6. The difference in average butanol yields between each condition. In red, conditions with significant differences according to **HSD=12.3970**

	C. pasteurianum	[0 mM fumarate]	[2.5 mM fumarate]	[10 mM fumarate]	[40 mM fumarate]
C. pasteurianum		47.0	55.9	29.9	110.0
[0 mM fumarate]			8.9	17.1	63.0
[2.5 mM fumarate]				26.0	54.1
[10 mM fumarate]					80.1
[40 mM fumarate]					

Table S7. ANOVA for 1,3-PDO yields

Groups	*No. samples*	*Sum*	*Average*	*Variance*
C. pasteurianum	3	326.3923817	108.7974606	76.11772245
[0 mM fumarate]	3	523.0172164	174.3390721	14.50915879
[2.5 mM fumarate]	3	536.6060286	178.8686762	12.93907463
[10 mM fumarate]	3	492.7398425	164.2466142	34.85184711
[40 mM fumarate]	3	685.0185991	228.339533	209.5269865

P-value = 1.65E-07

Table S8. The difference on average 1,3-PDO yields between each condition. In red, conditions with significant differences according to **HSD=20.8**

	C. pasteurianum	**[0 mM fumarate]**	**[2.5 mM fumarate]**	**[10 mM fumarate]**	**[40 mM fumarate]**
C. pasteurianum		65.5	70.1	55.4	119.5
[0 mM fumarate]			4.5	10.1	54.0
[2.5 mM fumarate]				14.6	49.5
[10 mM fumarate]					64.1
[40 mM fumarate]					

Table S9. Final acetate concentration

C. pasteurianum	**[0 mM fumarate]**	**[2.5 mM fumarate]*[a]**	**[10 mM fumarate]*[b]**	**[40 mM fumarate]*[c]**
15.1	13.3	15.3	17.7	25.1
16.4	13.0	14.9	18.7	27.6
15.8	13.2	14.6	17.0	26.1

*The final concentrations were corrected by adding the theoretical acetate consumed according to the succinate produced. Assumptions were based on the *G. sulfurreducens* pure cultures with 40 mM fumarate where 12.9 ± 0.29 mM acetate were consumed and 37.5 ± 0.97 mM succinate were produced. a: +0.8 mM acetate; b: +3.1 mM acetate; c: +10.9 mM acetate.

Table S10. ANOVA for final acetate concentrations.

Groups	*No. samples*	*Sum*	*Average*	*Variance*
C. pasteurianum	3	47.2560976	15.7520325	0.39274508
[0 mM fumarate]	3	39.5325203	13.1775068	0.02868846
[2.5 mM fumarate]	3	44.7949864	14.9316621	0.14927564
[10 mM fumarate]	3	53.3887534	17.7962511	0.76378255
[40 mM fumarate]	3	78.7196477	26.2398826	1.55328909

P-value = 1.13E-08

Table S11. The difference in average acetate final concentrations between each condition. In red, conditions with significant differences according to **HSD=1.9**

	C. pasteurianum	**[0 mM fumarate]**	**[2.5 mM fumarate]**	**[10 mM fumarate]**	**[40 mM fumarate]**
C. pasteurianum		2.6	0.8	2.0	10.5
[0 mM fumarate]			1.8	4.6	13.1
[2.5 mM fumarate]				2.9	11.3
[10 mM fumarate]					8.4
[40 mM fumarate]					