Supplementary Materials: Production of 1,3-Propanediol from Pure and Crude Glycerol Using Immobilized *Clostridium Butyricum*

Igor Dolejš, Monika Líšková, Vladimír Krasňan, Kristína Markošová, Michal Rosenberg, Fabio Lorenzini, Andrew C. Marr, Martin Rebroš*

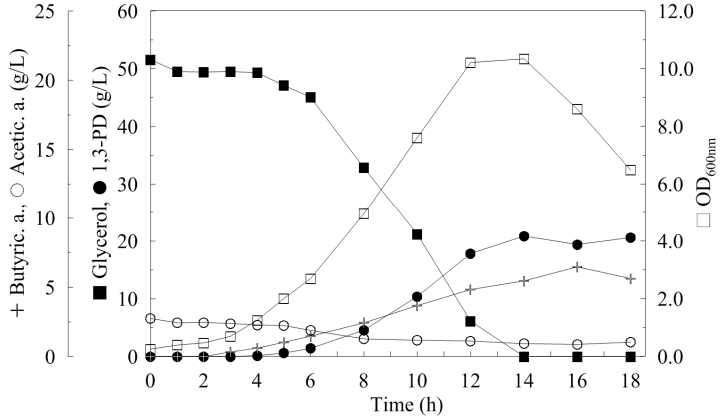

Figure S1. Batch fermentation with free cells of *C. butyricum* performed with initial concentration of 51.6 g/L of pure glycerol.

Table S1. Comparison of published data on fermentation of crude and pure glycerol by strains of *C. butyricum*.

Type of process	Strain	Type; Conc. of glycerol (g/L)	Conc. of 1,3-PD (g/L)	Yield (g/g)	Productivity (g/L·h)	Reference
Batch	*C. butyricum* NRRL B-23495	pure; 20.0	11.3	0.58	0.7	Metsoviti et al., 2012 [49]
Batch	**C. diolis* DSM 15410	pure; 54.2	25.8	*0.54	0.7	Kaur et al., 2012 [4]
Batch	*C.butyricum* VPI 3266	pure; 39.6	7.4	*0.48	0.3	González-Pajuelo et al., 2004 [43]
Batch	*C.butyricum* JKT37	pure; 40 ± 0.9	19.6 ± 0.3	*0.50	2.8	Tee et al., 2017 [50]
Repeated batch	*C.butyricum* DSM 4278	pure; 45.5 ± 0.7	17.0 ± 0.6	0.40 ± 0.02	6.8 ± 0.2	(This study)
Batch	*C. butyricum* F2b	crude; 90.0	47.1	*0.53	1.1	Papanikolaou et al., 2004 [48]
Batch	*C.butyricum* VPI 3266	crude; 44.1	8.3	*0.42	0.3	González-Pajuelo et al., 2004 [43]
Batch	*C. butyricum* F2b	crude; 50.0	27.5	0.55	*1.6	Papanikolaou et al., 2004 [48]
Batch	*C. butyricum* NRRL B-23495	crude; 55.0	32.3	0.59	*0.9	Metsoviti et al., 2012 [49]

Repeated batch	*C.butyricum* DSM 4278	***crude; 40.0 ± 1.2	12.6±0.9	0.35 ± 0.02	3.5 ± 0.3	(This study)
Fed-batch	*C. butyricum* VPI 1418	crude; 125.6	67.9	0.55	0.8	Chatzifragkou et al., 2011 [19]

*values calculated from available data; ***C. diolis* DSM 15410 (formerly *C. butyricum* DSM 5431); ***crude glycerol produced from rapeseed oil.

Table S2. Comparison of repeated batch fermentation performed by entrapped cells, cells spontaneously immobilised on the surface of PVA particles, and free cells, using pure or crude glycerol produced from rapeseed oil (RO) and used cooking oil (UCO).

	Pure glycerol				Crude glycerol		
	Free cells	Empty particles with adsorbed cells	Immobilised cells		Free cells	Immobilised cells	
			Repeated batch No. 18-27	Repeated batch No. 28-38	RO	UCO	RO
Initial glycerol (g/L)	51.6	45.0 ± 1.6	45.5 ± 0.7	70.4 ± 1.9	50.9	20.0 ± 0.2	40.0 ± 1.2
Repetitions	1	20	10	11	1	5	15
Residual glycerol (g/L)	0	2.4 ± 1.6	2.9 ± 0.6	2.0 ± 1.4	4.8	0.3 ± 0.2	4.0 ± 1.4
Consumed glycerol (g/L)	51.6	42.6 ± 2.0	42.5 ± 0.9	67.5 ± 1.4	46.1	19.7 ± 0.7	35.9 ± 1.8
Duration (h)	14.0	3.6 ± 0.2	2.5 ± 0.1	5.0 ± 0.1	19.0	2.5 ± 0	3.6 ± 0.4
$OD_{600\ nm}$	10.3	8.7 ± 1.6	6.4 ± 1.1	9.8 ± 0.4	-	-	-
1,3-PD (g/L)	20.9	15.3 ± 0.8	17.0 ± 0.6	28.3 ± 0.6	17.7	5.9 ± 0.2	12.6 ± 0.9
Butyric acid (g/L)	5.5	5.7 ± 0.4	5.1 ± 0.3	8.6 ± 0.4	6.6	3.3 ± 0.3	5.0 ± 0.5
Lactic acid (g/L)	5.9	4.5 ± 0.3	3.8 ± 0.3	5.1 ± 0.7	9.3	0	3.3 ± 0.4
Yield (g/g)	0.40	0.36 ± 0.01	0.40 ± 0.02	0.42 ± 0.01	0.38	0.30 ± 0.01	0.35 ± 0.02
Productivity (g/L·h)	1.5	4.2 ± 0.1	6.8 ± 0.2	5.6 ± 0.1	0.9	2.3 ± 0.1	3.5 ± 0.3

Table S3. Composition of crude glycerol produced from used cooking oil (UCO) and rapeseed oil (RO).

	UCO	RO
Fatty acids and fatty acid esters % (w/w)	13.2	0.4
Methanol % (w/w)	1.7	16.1
Density (g/mL)	1.2	1.1
Water content % (w/w)	3.4	0.4
Ash content % (w/w)	4.7	2.8
Glycerol % (w/w)	76.9	81.1