Supplementary information

Citrobacter werkmanii, a new candidate for the production of 1,3propanediol: strain selection and carbon source optimization

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1. PDO inhibition tests: Residual glycerol concentration

Table S1: Residual glycerol concentration (g/L) growing *C. braakii* DSM17579 (CB17579), *C. freundii* NRRL B-2644 (CF2644), *C. braakii* DSM30040 (CB30040), *C. werkmanii* DSM17579 (CW17579), and *C. freundii* NRRL B-2643 (CF2643) on different PDO concentrations (0-60 g/L) in shake flasks under anaerobic conditions. The values are the average of two independent experiments with their corresponding standard deviation. BDL = below the detection limit; ND = not determined.

Strain	0 (g/L)	20 (g/L)	40 (g/L)	60 (g/L)
CB17596	10.77±0.25	15.57±0.15	14.18±0.21	ND
CF2644	BDL	12.45±0.02	14.01±0.33	ND
CB30040	BDL	0.21±0.16	12.31±0.18	10.82±0.21
CW17579	BDL	0.13±0.01	0.44±0.01	11.18±0.31
CF2643	BDL	BDL	11.09±0.08	10.87±0.21

2. Glycerol inhibition: 3-HPA concentration

During the substrate inhibition tests, it is noted that the growth rate decreases significantly going from 20 g/L to 60 g/L glycerol, after which it stagnates. A first possible explanation is the presence of the toxic intermediate 3-hydroxypropionaldehyde (3-HPA). Tests have revealed that growth of *C. werkmanii* DSM17579 ceases at a concentration of 2.96 g/L 3-HPA (unpublished results). However, this concentration is not reached during the inhibition experiments, as can be noticed in Fig. S1. The method to determine the 3-HPA concentration is described in the Experimental section.



Fig. S1: 3-HPA profile of the batch fermentations on bioreactor scale growing *Citrobacter werkmanii* DSM17579 on different initial glycerol concentrations : (A) 20 g/L (\bullet) and 40 g/L (∇); (B) 60 g/L; (C) 80 g/L and (D) 100 g/L initial glycerol concentration. The errors represent the standard deviation calculated from 2 independent experiments.

3. Glycerol inhibition: Growth of C. werkmanii DSM17579 with mixture of acids

A second possible explanation for the reduced growth rate during the substrate inhibition tests is the formation of byproducts, such as succinate, acetate, and ethanol. Therefore, *C. werkmanii* DSM17579 was inoculated in a shake flasks with 15 g/L glycerol and 10 g/L acetate, 4 g/L lactate, 6 g/L succinate, 5 g/L formate, and 7 g/L ethanol, and grown anaerobically during 48 h. The results of this growth experiments are depicted in Table S2.

Strain	Start OD _{600 nm}	OD _{600 nm} 24h	OD _{600 nm} 48h
C. werkmanii 1	0.185	0.255	0.190
C. werkmanii 2	0.220	0.180	0.185

Table S2: Growth of *C. werkmanii* DSM17579 in shake flasks under anaerobic conditions with 15 g/Lglycerol and 10 g/L acetate, 4 g/L lactate, 6 g/L succinate, 5 g/L formate, and 7 g/L ethanol

4. Sugars as only carbon source: L-fucose as inducer of D-arabinose metabolization pathway

C. werkmanii DSM17579 is unable to grow on D-arabinose as sole carbon source, but when 1 mM Lfucose is added as inducer, the strain is able to grow and consume D-arabinose (Table S3). Therefore, we have postulated that the degradation pathway of D-arabinose is analogous to the one of *E. coli* K12 MG1655.

Table S3: Growth rate (h^{-1}), residual concentration D-arabinose (mM) and produced succinate, acetate, and ethanol concentration (mM) of *C. werkmanii* DSM17579 grown in shake flasks under anaerobic conditions on D-arabinose with or without 1 mM L-fucose. The values are the average of two independent experiments with their corresponding standard deviation. BDL = below the detection limit.

		Residual	Product concentration (mM)		
Condition	Growth rate	concentration (mM)			
	(h⁻¹)	D-arabinose	Succinate	Acetate	Ethanol
Without L-fucose	BDL	69.30±1.44	BDL	BDL	BDL
With L-fucose	0.07±0.01	BDL	8.69±0.38	77.31±0.42	34.36±1.89

5. Sugars as co-substrate: NADH-dependent 1,3-propanediol dehydrogenase

In natural PDO-producing micro-organisms, 3-HPA can be converted either by a NADH-dependent 1,3-propanediol dehydrogenase (PDODH) or a NADPH-dependent 1,3-propanediol oxidoreductase (PDOR). We have postulated that the NADH-dependent PDODH is responsible in *C. werkmanii* DSM17579 as the ethanol concentration is reduced comparing the sugars as only carbon source with the sugars as co-substrate, except for the Polyol sugars. These results are depicted in Fig. S2.



Fig. S2: Effect of glycerol on the ethanol production (M) by *C. werkmanii* DSM17579. The errors represent the standard deviation calculated from 2 independent shake flask experiments with cultivation medium containing either the sugars as only carbon source or 0.33 molar ratio co-substrate/glycerol. The Polyols are circled.

6. Sugars as co-substrate: Glycerol is still used for growth

Table S4: Final biomass concentration measured as $OD_{600 \text{ nm}}$ for the growth of *C. werkmanii* DSM17579 in shake flasks under anaerobic conditions in cultivation medium with 0.33 mol co-substrate/mol glycerol. The values are the average of two independent experiments with their corresponding standard deviation.

Co-substrate	OD _{600 nm}
D-glucose	1.58±0.05
D-maltose	1.02±0.01
D-mannose	1.65±0.01
D-fructose	1.72±0.02
L-fucose	1.19±0.08
L-rhamnose	0.96±0.06
DHA	0.78±0.06
D-sorbitol	1.13±0.02
D-mannitol	1.45±0.04
D-galactose	1.67±0.01
L-arabinose	1.34±0.08
D-arabinose	1.33±0.05
D-ribose	1.45±0.05
D-xylose	1.43±0.02
D-sucrose	0.98±0.01
Glycerol	1.35±0.04

7. Growth of *C. werkmanii* DSM17579 on crude glycerol derived from a biodiesel preparation using rapeseed oil

Table S5: Effect of pure and crude glycerol as substrate on the growth rate (h⁻¹), PDO titer (mM), and yield on glycerol of PDO (mol/mol) of *C. werkmanii* DSM17579 grown in cultivation medium under anaerobic conditions. The values are the average of two independent experiments with their corresponding standard deviation.

Substrate	Growth rate (h^{-1})	PDO titer (mM)	Yield (mol PDO/mol glycerol)
Pure Glycerol	0.33±0.02	141.93±2.93	0.64±0.02
Crude Glycerol	0.39±0.02	126.50±4.49	0.58±0.03