frontiers

Supplementary Material: Concentration pulse method for the investigation of transformation pathways in a glycerol-fed bioelectrochemical system

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1 STEP CHANGES IN RESIDENCE TIME

The substances concentration pulse experiments were selected based on previous experimental runs. Intermediates that were detected in the previous experiment were injected for the concentration pulses. The development of current and concentrations over time for a previous experiment with an inlet concentration of
I -> L $7.55\,\mathrm{mmol\,l^{-1}}$ is depicted in figure S1. After a lag phase, the current increases rapidly, reaches a maximum of
$0.65\,\mathrm{mA\,cm^{-2}}$, and falls back to a steady state value of $0.3\,\mathrm{mA\,cm^{-2}}$ on day 13. The peak current density is comparable to pure *Geobacter* spp. biofilms metabolizing acetate Liu et al. (2015). Propionate and 1,3-propanediol are the most prominent intermediates in this steady state. From day 21 onwards, the residence time was reduced stepwise from 21.8 h down to 10.8 h and 5.4 h. While the current slightly increased upon each reduction of the residence time, the concentrations of intermediates only changed when the residence time was reduced

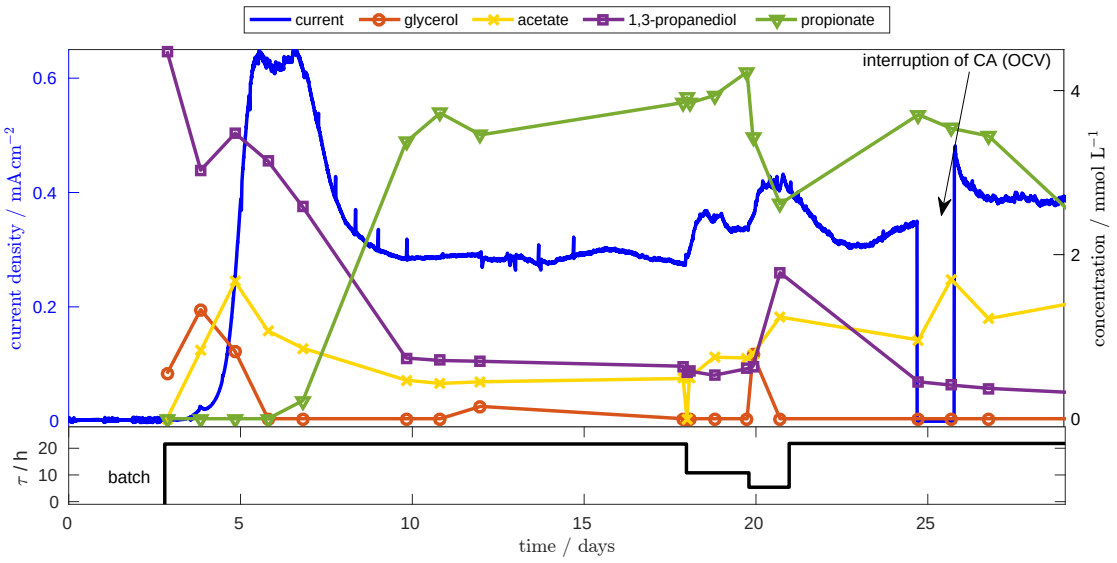


Figure S1: Current and concentrations over time during an experiment with a glycerol inlet concentration of 7 mM. Residence time was stepped from 21.6 to 10.8 and 5.4 h by adjusting the flow rate.

to 5.4 h. On day 27, the current was stepped down to zero (open circuit conditions) for one day. After these changes, the system was reverted to the original operational conditions. All in all, the results of this experimental series do not shed much light on the mechanisms of glycerol electro-oxidation. They did help, however, to identify intermediates for the pulse experiments and to choose residence time and inlet concentration for the experiments reported in the main part of the manuscript.

2 REPETITION OF THE GLYCEROL CONCENTRATION PULSE

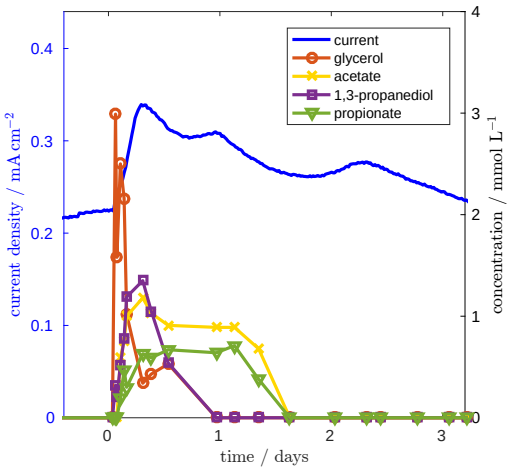


Figure S2: Concentration response to a glycerol concentration pulse at t=0. Residence time is 28.8 h, glycerol inlet concentration 1.8 mM.

3 PULSES AT OCV

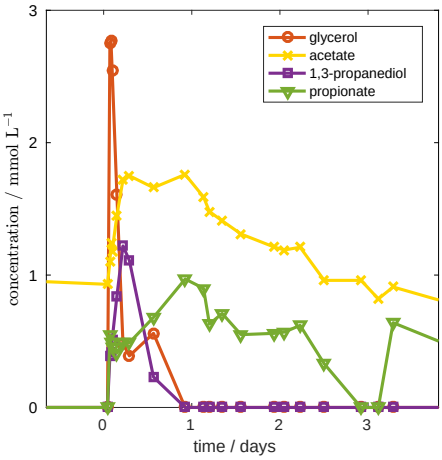


Figure S3: Concentration response to a glycerol concentration pulse at t=0 under open circuit conditions. Residence time is 28.8 h, glycerol inlet concentration 1.8 mM.*

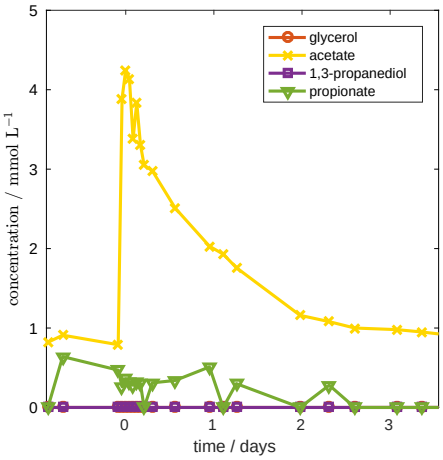


Figure S4: Concentration response to an acetate concentration pulse at t=0 under open circuit conditions. Residence time is 28.8 h, glycerol inlet concentration 1.8 mM. Glycerol and 1,3-propanediol were not detected. Propionate probably results from the preceding glycerol pulse.*

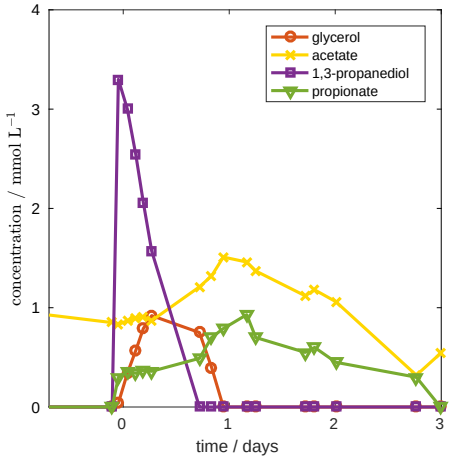
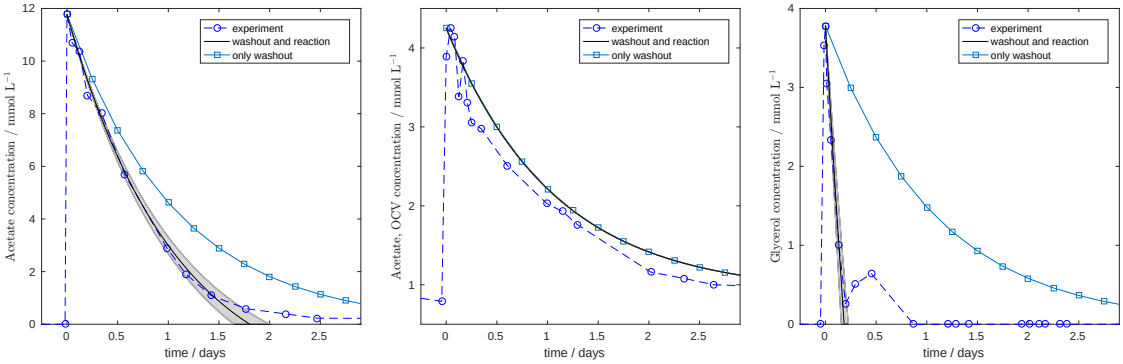


Figure S5: Concentration response to a 1,3-propanediol concentration pulse under open circuit conditions at t=0. Residence time is 28.8 h, glycerol inlet concentration 1.8 mM.

* = Quantitative interpretation of the proprionate concentration values in figure S3 and S4 should only be undertaken with great care since the zero concentration values indicate that an error might have occurred in the sampling or HPLC analysis.

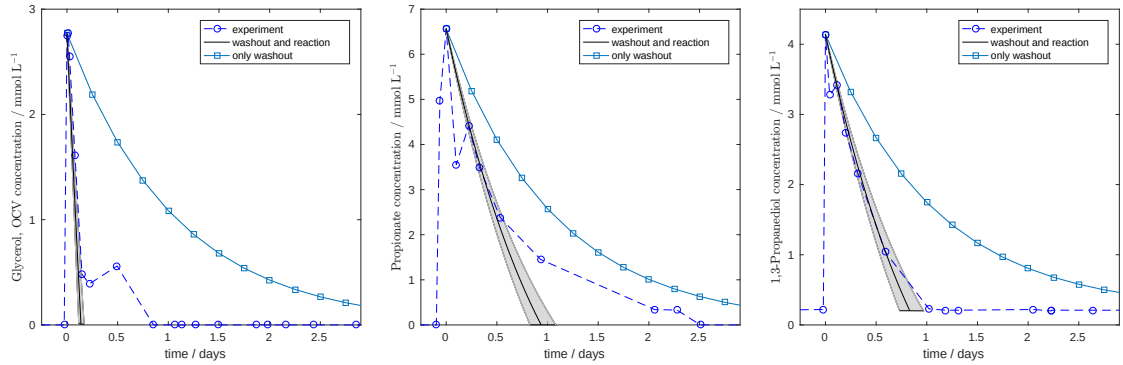
4 SIMULATED CONCENTRATION TRANSIENTS



a) acetate:
$X \cdot q_{\max}$ =0.4mM/day

b) acetate, OCV:
$X \cdot q_{\max}$ =0.0mM/day

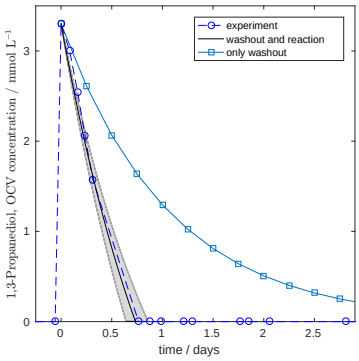
c) glycerol:
$X \cdot q_{\max}$ =3mM/day



d) glycerol OCV:
$X \cdot q_{\max}$ =3mM/day

e) propionate:
$X \cdot q_{\max}$ =0.7mM/day

f) 1,3-propanediol:
$X \cdot q_{\max}$ =0.5mM/day



g) 1,3-propanediol OCV:
$X \cdot q_{\max}$ =0.5mM/day

Figure S6: Experimental and simulated concentration transients for the pulsed component during the pulse experiments. Values of $X \cdot q_{\max}$ were adjusted to reproduce dynamic experiments. The gray corridor indicates the concentration transients for the respective value of $X \cdot q_{\max} \pm 20\%$, the propionate pulse response was not determined under open-circuit conditions

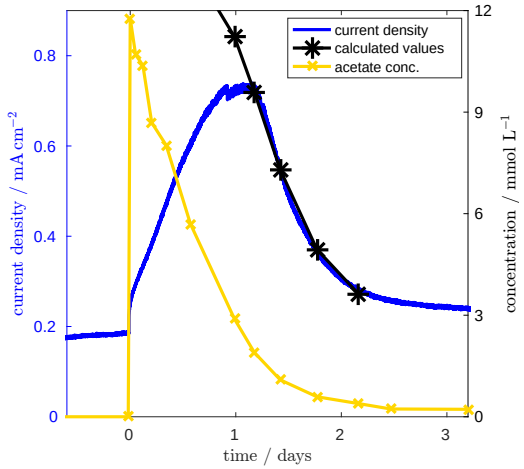


Figure S7: Determination of K_S for the biofilm from the acetate concentration pulse. The line with the asterisk markers is calculated by ~~I~~ $=$ ~~I_{max}~~ $\cdot\, c_{\text{Acetate}}/(c_{\text{Acetate}} + K_S)$ with $K_S = 1.4\,\text{mmol}\,\text{L}^{-1}$, and ~~$I_{max}$~~ $= 1.25\,\text{mA}\,\text{cm}^{-2}$. K_S and A were calculated from measured current and concentration values according to:
$\frac{\text{~~}I\text{~~}(t_1)}{\text{~~}I\text{~~}(t_2)} = \frac{\text{~~}I_{max}\text{~~}\cdot c_{\text{Acetate}}(t_1)}{(c_{\text{Acetate}}(t_1)+K_S)} \cdot \frac{(c_{\text{Acetate}}(t_2)+K_S)}{\text{~~}I_{max}\text{~~}\cdot c_{\text{Acetate}}(t_2)}$.

5 TURNOVER CV

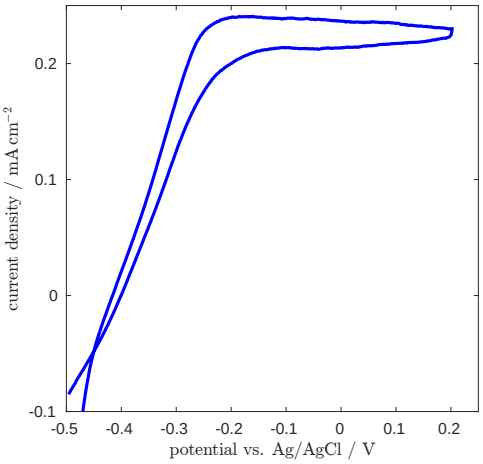


Figure S8: Turnover CV recorded before the concentration pulse experiments, showing that the current is not limited by the anode potential above -0.1 V. Residence time is 28.8 h, glycerol inlet concentration 1.8 mM, scan rate 1 mV/s.

Table S1: Intermediates detected in glycerol-fed BES in literature. Note that an empty field includes the cases "not found" as well as "not determined" and "not reported"

	COD	glycerol	valerate	butyrate	1,3-PDO	lactate	propionate	ethanol	acetate	formate	CO_2	CH_4	H_2	culture	type	mode	others
Selembo et al. (2009)		x			x		x		x		x	x	x	mixed	MEC	B	
Montpart et al. (2015)	x	x					x		x			x	x	mixed	MEC/MFC	FB	
Zhou et al. (2013)		x	x	x	x		x	x	x	x	x	x	x	mixed	EF	C	*
Kumar and Venugopalan (2015)		x				x		x	x	x	x		x	*Enterobacter aerogens*	MEC	B	
Moscoviz et al. (2017)		x			x	x	x	x	x	x				mixed	EF	B	**
Chookaew et al. (2014)	x	x	x	x			x		x				x	mixed	MFC/MEC	B	***
Sharma et al. (2011)		x		x	x			x	x					mixed	MFC	B	
Escapa et al. (2009)		x		x	x		x		x				x	mixed	MEC	C	
Kim et al. (2016)		x												*Shewanella Oneidensis & Klebsiella pneumonae*	MFC	B	
Feng et al. (2011)	x													mixed	MFC	B	
Tremouli et al. (2016)	x													mixed	MFC	B	
Chignell, Jeremy F, Liu (2011)												x	x	mixed	MEC	B	
Nimje et al. (2011)														*Bacillus subtilis*	MFC	B	
This work		x		x	x		x		x		(x)	(x)		mixed	MFC	C	

* = hexanoic acid, ** = succinate, *** = isocaproic acid, caproic acid, octanoic acid and isovalerate, EF = electro-fermentation,
MFC = microbial fuel cell, MEC = microbial electrolysis cell, 1,3-PDO = 1,3-propanediol, B = batch, C = continuous, FB = fed-batch

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