Supporting information

Continuous long-term electricity-driven bioproduction of carboxylates and isopropanol from CO_2 with a mixed microbial community

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Methods S1. Analyses of alcohols by GC

Analysis of alcohols from C1 to C4 was done using a gas chromatography (CP-3800 + CP-8400 autosampler, Varian®, The Netherlands) equipped with a PoraPlot Q CP7550-PT column (10 m x 0.32 mm x 10 μm; Agilent, Belgium) and a flame ionization detector (FID) based on a protocol described in Coma et al. [1]. One mL of filtered (0.45 μm) aqueous samples was added to a vial containing 100 μL of freshly prepared 20 % acetone as an internal standard (14.4 g L^{-1}). Prepared sample (0.5 μL) was injected at 200 ºC with a split ratio of 30. The oven temperature was held at 140 ºC for 2.1 minutes and then increased by 10ºC min^{-1} from 140 ºC to 165 ºC and by 30 ºC min^{-1} from 165 ºC to 200 ºC where it was kept for 2.23 min. The carrier gas was helium with an initial pressure of 7 Psi for 2.10 min, increased by 0.5 Psi min^{-1} to 8 Psi and by 2.5 Psi min^{-1} to 10 Psi where it was held during 3.09 min. The FID temperature was set at 230 ºC. Hydrogen and synthetic air flows were set at 30 mL min^{-1} and 300 mL min^{-1}, respectively.

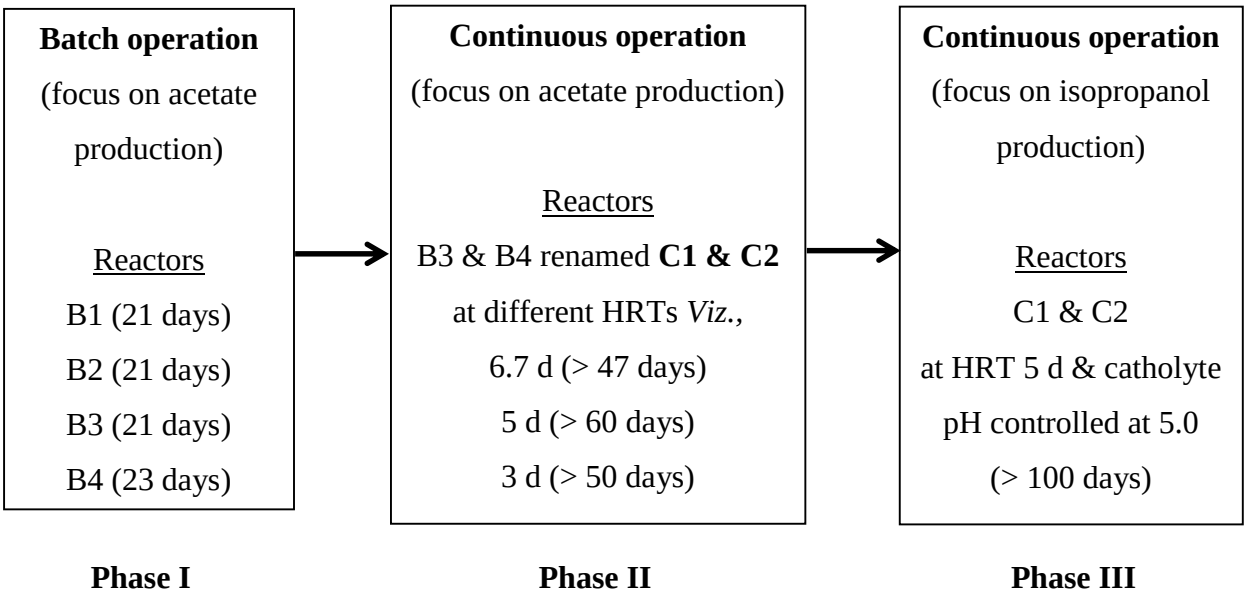


Figure S1. Overall experimental scheme divided into three phases. HRT – Hydraulic retention time

Table S1. Comparison of key cathodic electrochemical and acetate production parameters of microbial electrosynthesis reactors operated in a continuous flow mode.

Microbial inoculum		**Cathode material**	**Cathodic electrochemical parameters**		**Acetate production rates**		**Electron recovery in acetate (%)**	**Reference**
			$J_{applied/produced}$ (A m^{-2})[#]	**$E_{cathode}$ (V vs SHE)**	**g L^{-1} d^{-1}**	**(g m^{-2} d^{-1})[#]**		
Pure cultures	*Sporomusa ovata*	Graphite stick	0.208	-0.4	0.045	1.3	86 ± 21	[2]
	Sporomusa sphaeriodes	Graphite stick	0.017	-0.4	0.002	0.06	84	[3]
	Morella thermoacetica	Graphite stick	0.009		0.003	0.1	84	
	Clostridium ljungdhalii	Graphite stick	0.029		0.003	0.1	72	
	Sporomusa ovata	Ni-coated graphite stick	0.63	-0.4	0.067	3.33	82 ± 14	[4]
	Sporomusa ovata	Carbon cloth + chitosan	0.475 ± 0.018	-0.4	0.064 ± 0.016	2.70 ± 0.66	86 ± 12	[5]
		Carbon cloth + Cyanuric chloride	0.451 ± 0.079	-0.4	0.057 ± 0.014	2.42 ± 0.59	81 ± 16	
	Sporomusa ovata	Graphite disk (membraneless reactors)	0.17 ± 0.04	-0.66	0.099 ± 0.014	1.65 ± 0.24	105 ± 5	[6]
			0.46 ± 0.03	-0.81	0.128 ± 0.043	2.13 ± 0.71	54 ± 10	
		Graphite stick	1.7 ± 0.19	-0.74	0.315 ± 0.086	9.68 ± 2.66	89 ± 12	
Mixed cultures	Mix of anaerobic digester and retention basin effluents	Graphite granules	[$]12.3 ± 0.8 A m^{-3}	-0.6	0.024±0.003	2.7 ± 0.4[~]	28.9 ± 6.1	[7]*
	Enriched culture (from Labscale anode and algae UASB sludge)	Carbon felt	5	-1 ± 0.06	0.94 ± 0.04	18.8 ± 0.8	56 ± 2.3	This study**

[#]calculated per projected cathode surface; [$]based on the net cathode volume; [~]data provided by the authors; *HRT - 0.812 d; **HRT - 3.3 d

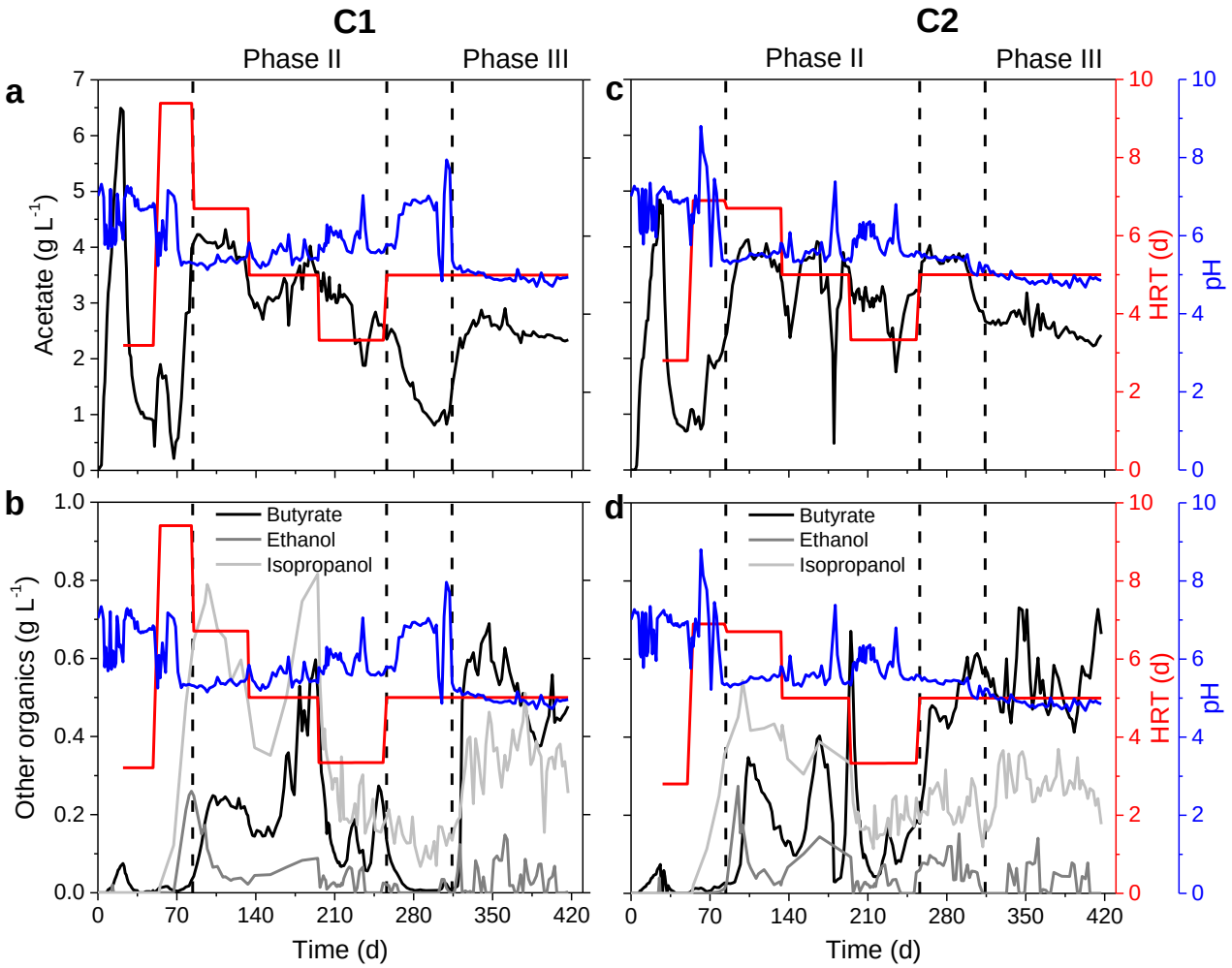


Figure S2. An overview of complete experimental run for reactors C1 (left panel; a & b) and C2 (right panel; c & d) illustrating acetate (top panel; a & c) and other organics (bottom panel; b & d) production profiles at different HRTs. Phase I – batch mode (followed by a continuous flow mode operation until day 84), Phase II – continuous flow mode operation at different HRTs (from day 84 to 256), Phase III – continuous flow mode operation at HRT 5 d and catholyte pH controlled at around 5.0 (from day 314 to 417). Before switching to the phase III experiments, both reactors were operated at HRT of 5 d with normal pH feed (7.6) in order to restore their production profiles.

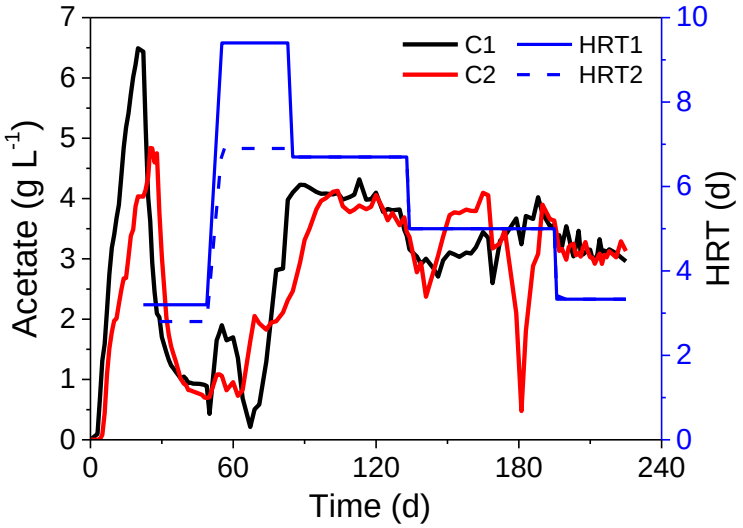


Figure S3. Continuous acetate production profiles for reactors C1 (from day 22) and C2 (from day 27) operated at different HRTs. Initial switching from batch to continuous feed regime was done at a low HRT of 2.9±0.3 d, which clearly resulted in a rapid decrease in the acetate concentration in both reactors. This was mainly due to the dilution of the catholyte and also due to the washout of biomass as confirmed by OD observations. After operating these reactors at a higher HRT of >7 d for four weeks, they were then switched to different HRT regimes starting from 6.7 d to 3.3 d.

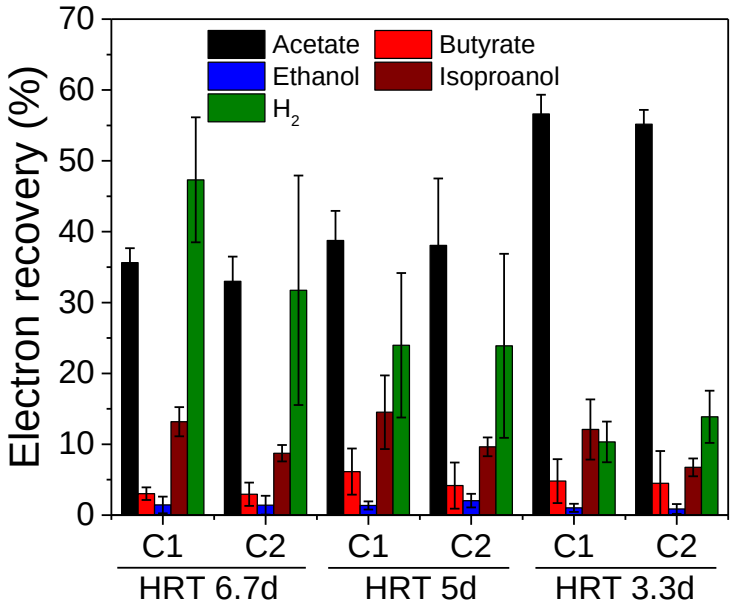


Figure S4. Electron recovery in different products (organic acids, alcohols and H_2) for reactors C1 and C2 operated in a continuous flow mode at different HRTs (experimental phase II).

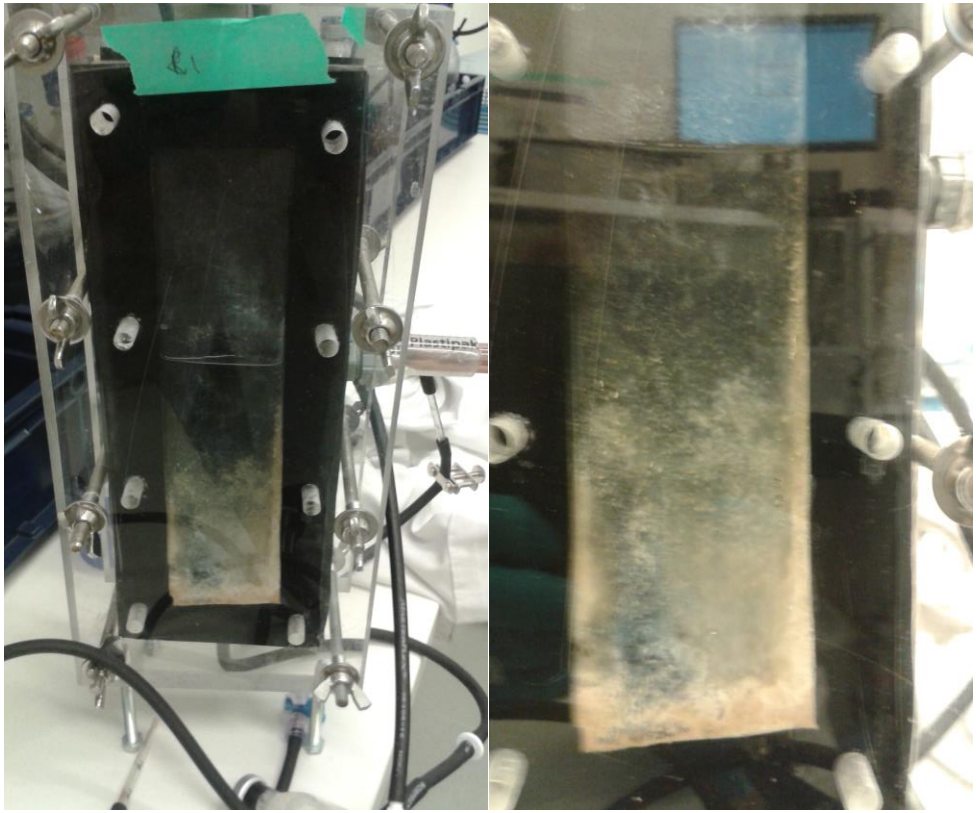


Figure S5. Photographic images of biocathodes showing (accumulated) biomass growth at the carbon felt electrodes. The images were captured at the end of the experiments.

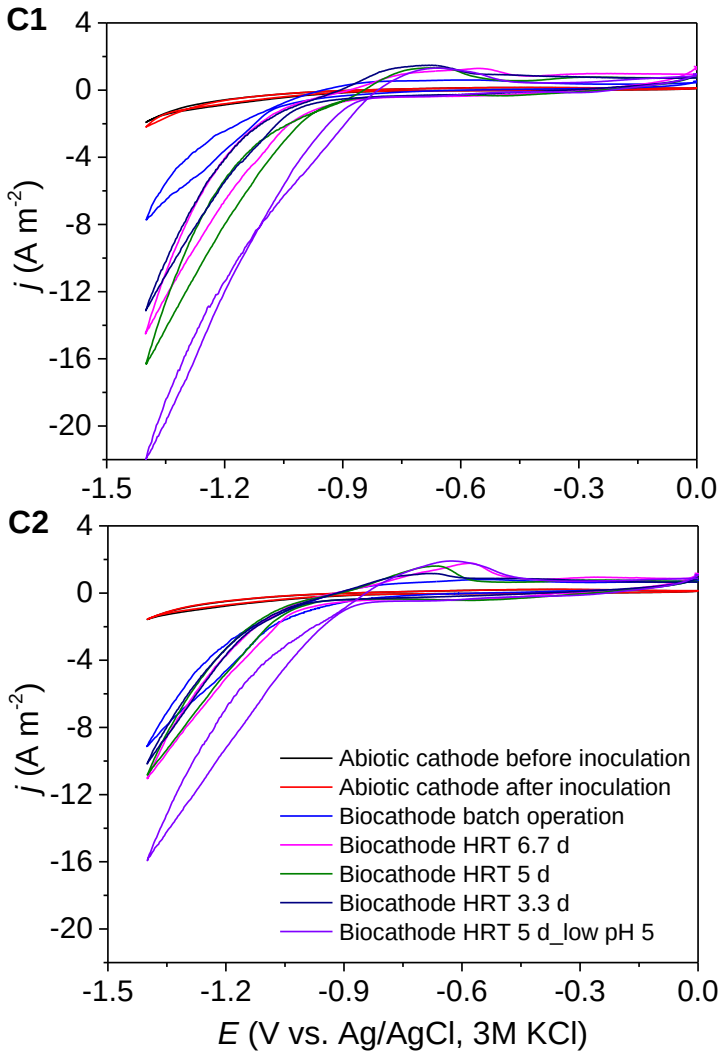


Figure S6. Cyclic voltammetry (CV) profiles of (bio)cathodes of reactors C1 and C2 recorded at different conditions – start of the experiment: before and immediate after inoculation (abiotic cathode controls), and at the end of batch operation and each HRT condition during continuous flow mode operation (biocathodes). Only the second cycle of two subsequent CVs is presented. Scan rate: 1 mV s^{-1}.

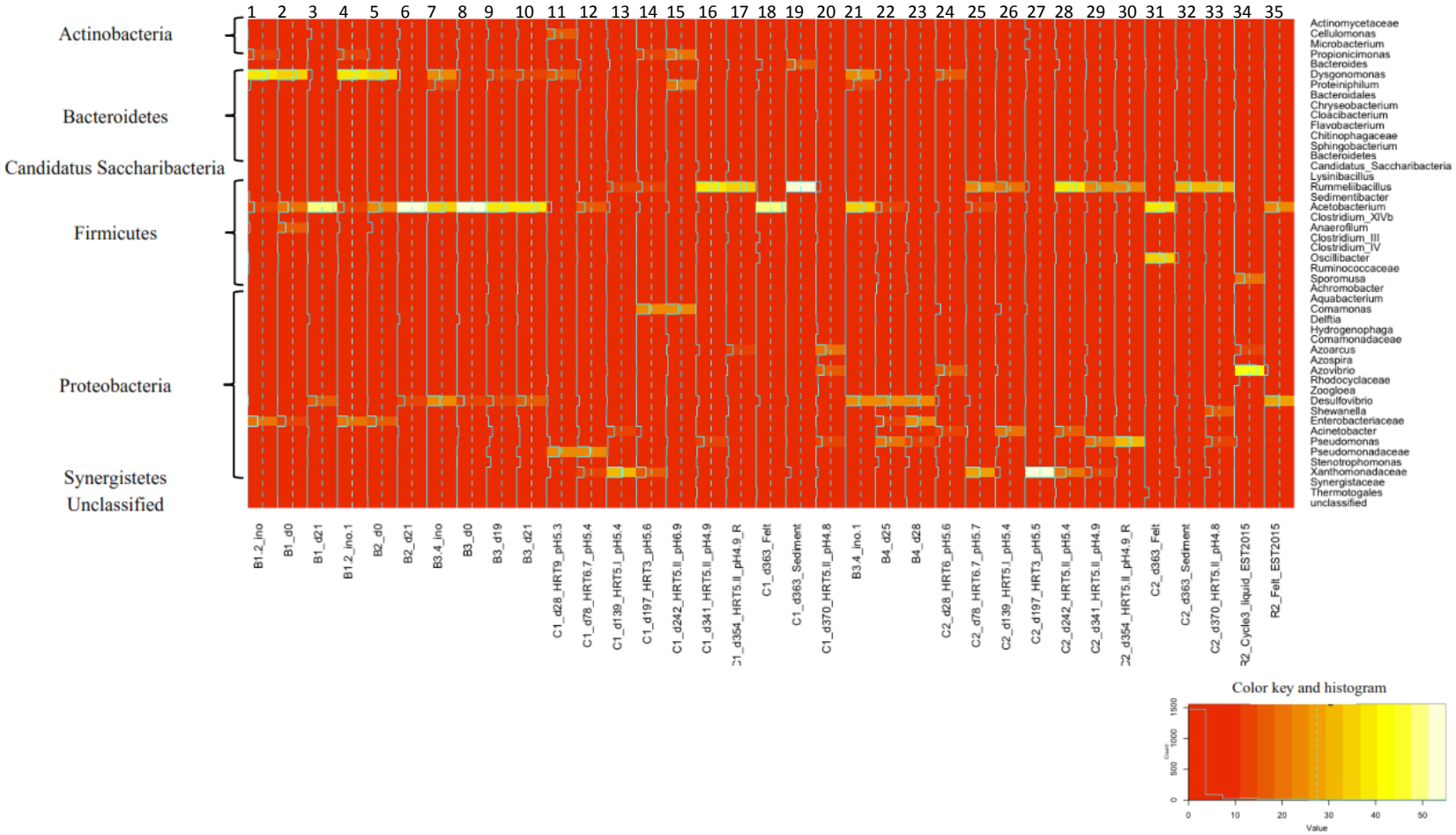


Figure S7. Heatmap representing the microbial communities of the 4 batch and 2 continues microbial electrosynthesis reactors. The left axis indicates phyla, the right axis indicates genus or the lowest taxonomic classification level above 1% relative abundance in at least 1 of the samples. Samples nomenclature: B: batch reactor, C: continuous reactor, ino: inoculum, d: day of sampling, HRT#: hydraulic residence time at the moment of sampling, pH#: pH at the moment of sampling, Felt: electrode attached community, Sediment: community of sedimented biomass. Roman numeral I and II indicate first and second time of HRT 5 days. The microbial communities of our previously published batch reactors (Patil et al. 2015) of the liquid (Lane 34) and electrode (Lane 35) are also represented. Note that for comparative purposes lane 1 contains the same information as lane 4, the same goes for lane 7 and 21.

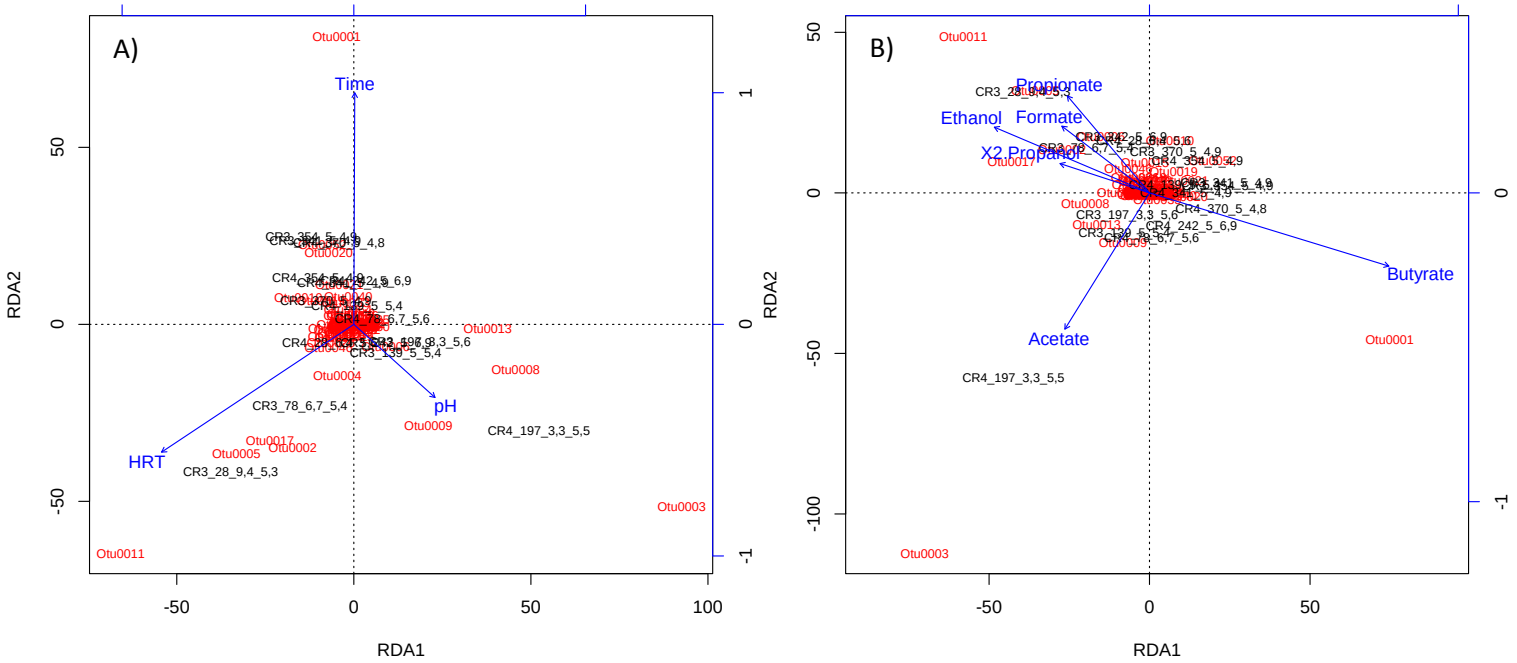


Figure S8. Redundancy analysis (RDA) highlighting the dissimilarities among the relative abundances of the bacterial communities in each samples obtained from continuous reactor operation (See also Figure S7) with A) the operational conditions Time, HRT and pH and B) organic components produced.

Correlation of the suspended microbial community with operational parameters by means of redundancy analysis indicated that the relative abundance of the genera *Comamonas*, *Azovibrio*, unclassified *Enterobacteriaceae* and unclassified *Xanthamonadaceae* were the most responsive to changes in HRT, whereas *Acetobacterium*, *Dysgonomonas*, and *Proteiniphilum* were the most responsive to changes in pH and the relative abundance of *Rummeliibacillus* was correlated with overall operating period (time).

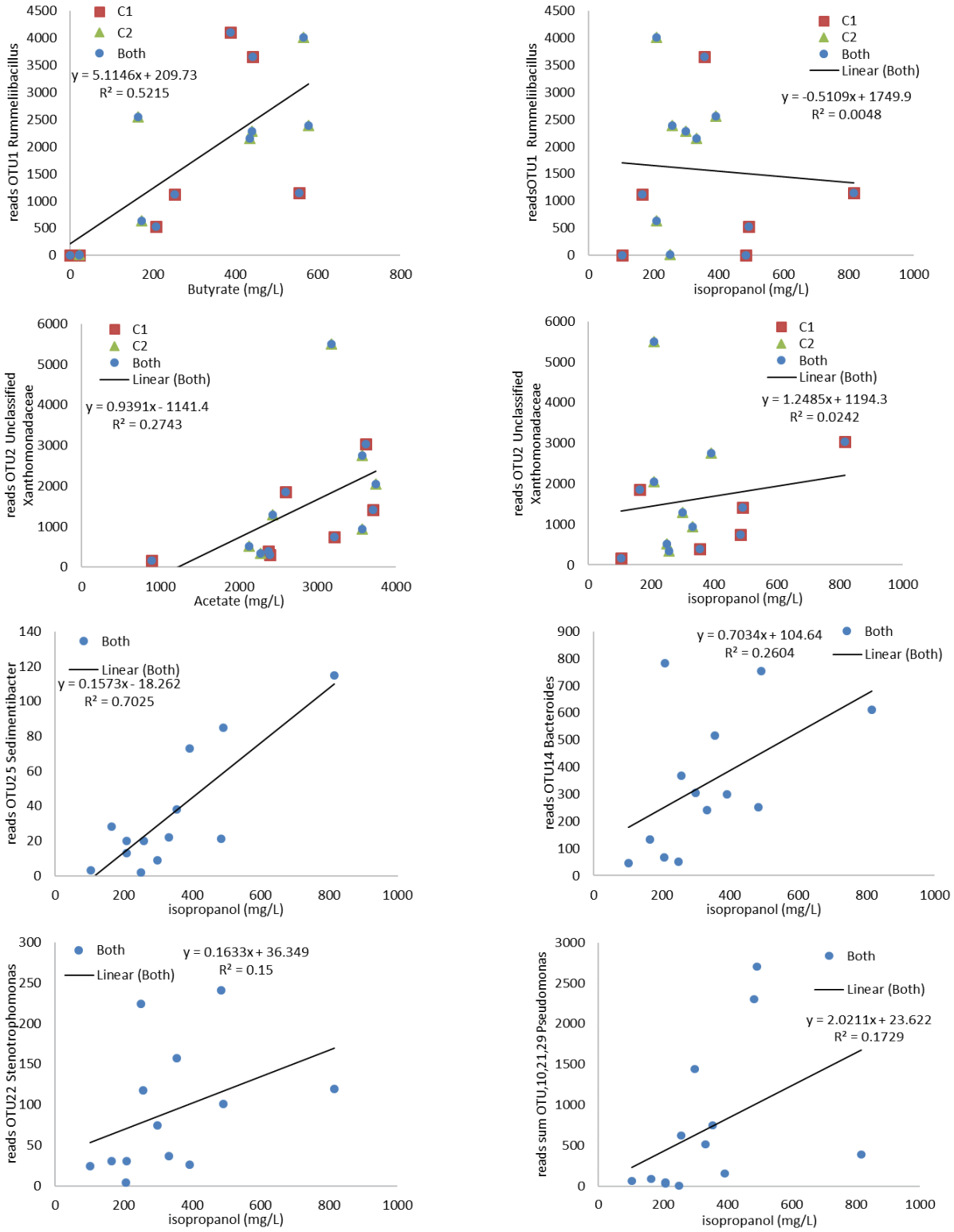


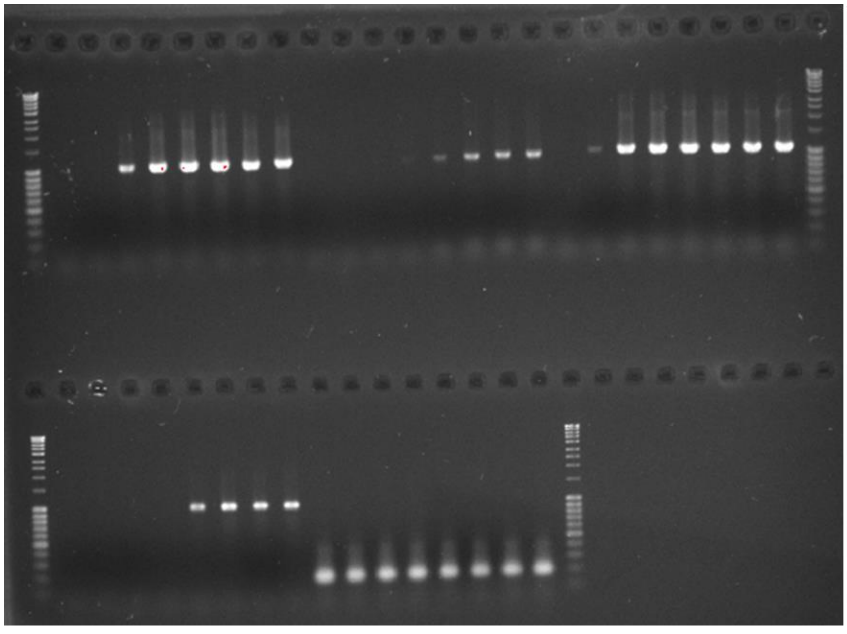
Figure S9. Selected correlations between product concentration and single OTU abundance. 'C1' or 'C2' indicates data obtained from the individual reactors, 'both', indicates data obtained from two reactors (C1 and C2).

Discussion S1. Specific sADH pcr

Several primer sets (Table S4) implicated for the detection of sADH of *Closterium beijerinckii* were tested using the mastermix and PCR protocol according to [8] and DNA from a pure culture of *C. beijerinckii* (DSM6423). The optimal annealing temperature was determined using a gradient PCR (Figure S9). None on the green conditions yielded a hit in on the DNA of the reactor community. This was partly confirmed by the Illumina analysis (Figure S6).

Table S3. Details of the primer sets used for the detection of sADH of *Closterium beijerincki.*

Code	Sequence	Tm (°C)	Reference
sADH1	5'- ATGAAAGGTTTTGCAATGCTAGGTATT-3'	58	sADH-1 [9]
sADH2	5'-TTATAATATAACTACTGCTTTAATTAAGTC-3'.	53	sADH-2
sADH3	5′-TAAGGAGGAACATATTTTATGAAAG-3′	51.9	P11 [10]
sADH4	5′-GTTATAATATAACTACTGCTTTAATTA-3′	49.5	P12
sADH7	5′- TTAGACTATTAAAGGAATATTTTTAAGG-3′	52.1	s-adh-for [11]
sADH8	5′- GTATAATCCTCCATGATCTATTATG-3′	51.5	s-adh-rev
sADH9	5'- ATGAAAGGTTTTGCAATGCTA -3'	52	P7 [12]
sADH10	5'- TTATAATATAACTACTGCTTTA -3'	44.5	P8



DNA: C. beijerinckii 1/10 dil

Lane #	Primers	T (°C)	size	ok?		Lane #	Primers	DNA	size	ok?
1	2 ul massruler ladder					1	2 ul massruler ladder			
2	sADH 1*2	65	1056	-		2	sADH 9*10	65	1056	-
3	sADH 1*2		1056	-		3	sADH 9*10		1056	-
4	sADH 1*2		1056	OK		4	sADH 9*10		1056	-
5	sADH 1*2		1056	OK		5	sADH 9*10		1056	-
6	sADH 1*2		1056	OK		6	sADH 9*10		1056	OK
7	sADH 1*2		1056	OK		7	sADH 9*10		1056	OK
8	sADH 1*2		1056	OK		8	sADH 9*10		1056	OK
9	sADH 1*2	45	1056	OK		9	sADH 9*10	45	1056	OK
10	sADH 3*4	65	1075	-		10	338f-GC * 518r	65	1075	OK
11	sADH 3*4		1075	-		11	338f-GC * 518r		236	OK
12	sADH 3*4		1075	-		12	338f-GC * 518r		236	OK
13	sADH 3*4		1075	-		13	338f-GC * 518r		236	OK
14	sADH 3*4		1075	/	weak	14	338f-GC * 518r		236	OK
15	sADH 3*4		1075	OK		15	338f-GC * 518r		236	OK
16	sADH 3*4		1075	OK		16	338f-GC * 518r		236	OK
17	sADH 3*4	45	1075	OK		17	338f-GC * 518r	45	236	OK
18	sADH 7*8	65	1122	-		18	2 ul massruler ladder			
19	sADH 7*8		1122	/	weak					
20	sADH 7*8		1122	/	2 products					
21	sADH 7*8		1122	/	2 products					
22	sADH 7*8		1122	/	2 products					
23	sADH 7*8		1122	/	2 products					
24	sADH 7*8		1122	/	2 products					
25	sADH 7*8	45	1122	/	2 products					
26	2 ul massruler ladder									

Figure S10. Results of annealing temperature optimization for detection of the sADH gene. Green primer/temperature combinations can be used to detect the presence of the sADH gene in the mixed community of the reactor.

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