Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Microbiome-based carboxylic acids production: from serum bottles to bioreactors

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Supplementary Methods: Determination of headspace gas composition

Gas chromatography analysis using a four channel 3000 Micro GC Gas Analyzer (INFICON, Cologne, Germany, specifications Table S1) with Argon and Helium as carrier gases and a thermal conductivity detector (TCD) was used to monitor changes in the headspace gas composition during anaerobic fermentation for medium-chain CA formation. External standard calibration allowed determining the proportion of H_2, O_2, N_2, CO_2, and CH_4 in the headspace. Produced gas was continuously collected in gas bags (barrier material: aluminium compound foil Hermann Nawrot AG, Wipperfuerth, Germany) with a reasonable volume (250 mL and 800 mL) in relation to the reaction volume for lab flasks and the bioreactors. For the serum bottles the gas sampling was carried out by overpressure release with a needle connected with a gas bag *via* a gas tight Tygon® tube (Fisher Scientific, Pittsburgh, USA). In order to collect a representative volume of headspace gas, the headspace gas of three replicates was sampled in one gas bag at each sampling. After sampling the tube of the gas bags were closed by a hose clamp and connected to the GC sampling port *via* a 3-way-valve which was further connected to a vacuum pump. Prior to analysis the tube connection was vacuumed to avoid any contribution of air (final oxygen levels between 0 and 5 %). After measurement the gas bags were vacuumed and reconnected to the respective reaction vessels.

Table S1: Specification of the GC-TCD measurements for the determination of the headspace gas composition.

Column	14 m Molecular sieve with 2 m Plot U pre-column, 1 µl backflush injector	8 m Plot Q, variable volume injector	8 m OV-1, 1.2 µm thick, variable volume injector	10 m Stabilwax column. 0.5 µm thick, variable volume injector
Sample inlet temperature [°C]	100	100	100	100
Injector temperature [°C]	100	100	100	100
Column temperature [°C]	120	60	60	60
Inject time [ms]	0	25	250	250
Run time [min]	3	3	3	3
Column pressure [psi]	25	20	20	15
Carrier gas	Argon	Helium	Helium	Helium

All columns and injectors were delivered by INFICON (Cologne, Germany).

Table S2: Details screening experiment.

The systematic screening of the microbiome for optimal cultivation conditions regarding ethanol and acetate concentrations and ratios was conducted in serum bottles.

Start conditions				
Ratio ethanol/ acetate	1.32	2.65	3.25	4.13
$c_{Ethanol}$ [mM]	311 ± 3	324 ± 8	322 ± 8	463 ± 1
$c_{Acetate}$ [mM]	235 ± 4	121 ± 7	102 ± 15	112 ± 11
Total carbon available [C-mM]	1092 ± 14	890 ± 30	848 ± 46	1150 ± 24
End conditions				
$c_{n\text{-Butyrate}}$ [mM]	68.3 ± 0.2	34.3 ± 0.7	23.4 ±0.5	11.8 ± 0.2
$c_{n\text{-Caproate}}$ [mM]	65.6 ± 1.0	58.7 ± 2.1	59.2 ± 0.57	6.2 ± 0.3
Ratio *n*-butyrate/ *n*-caproate	1.5	0.6	0.4	1.9
Total acid concentration [C-mM]	666.8	489.4	448.8	84.4
C recovery [%]	61.69	54.80	51.20	7.40

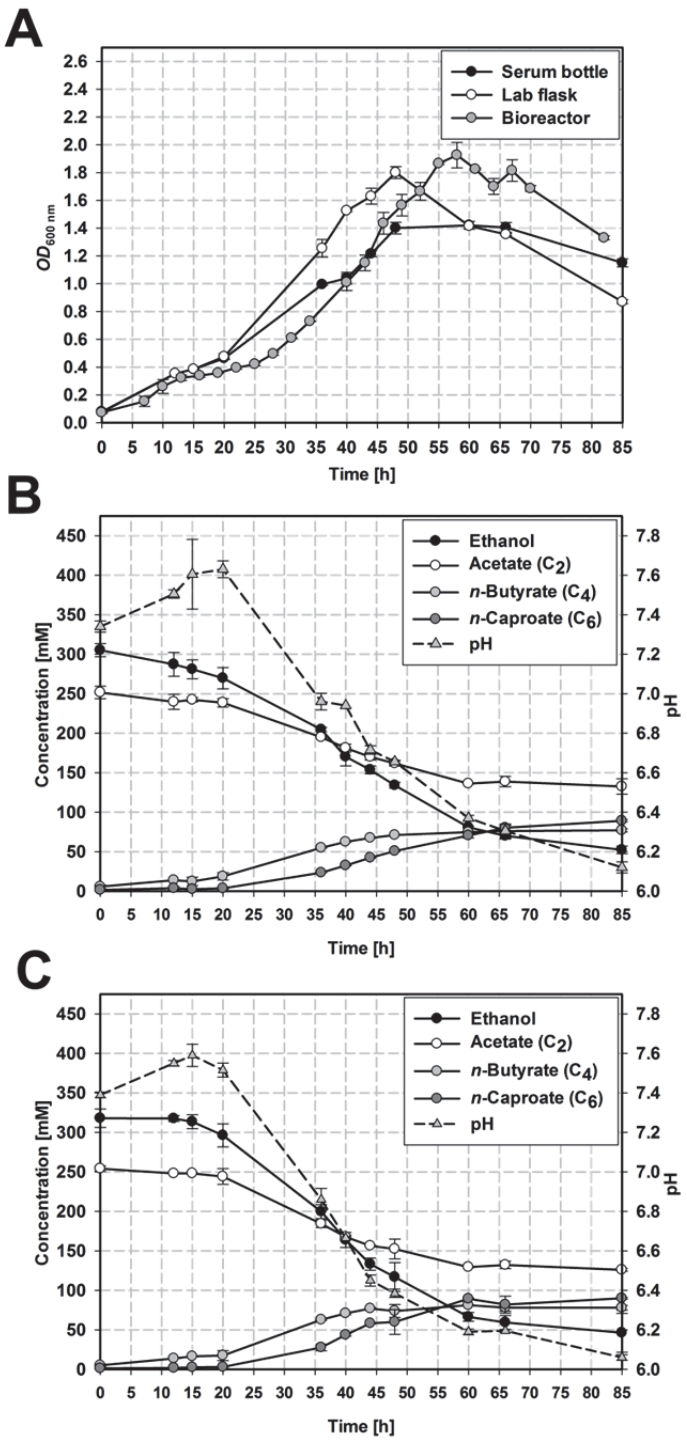

Figure S1: Microbiome-based medium-chain CA formation based on acetate and ethanol on different reactor scales. Comparative growth curves of microbiome development based on optical density (OD_{600nm}) measurements on serum bottle (110 ml, n=3), lab flask (440 ml, n=3) and conventional bioreactor (2.2 L, n=3) scale **(A)**. Accordant course of substrate conversion and production formation in serum bottles (**B)** and lab flasks(**C)**.

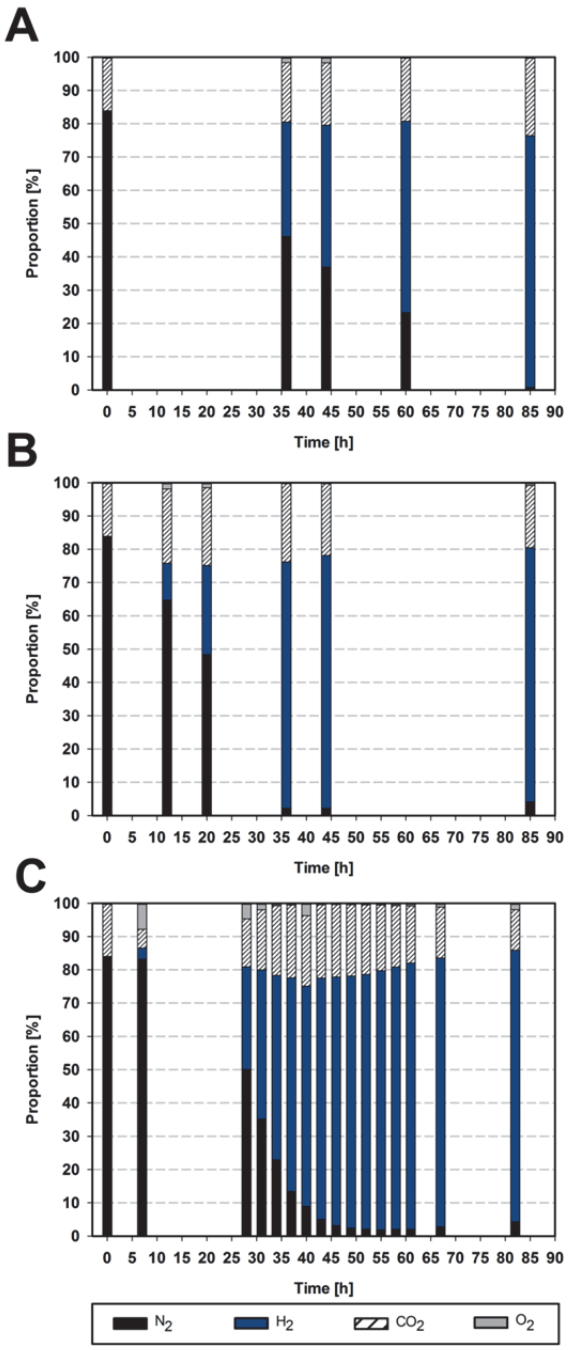

Figure S2: Change of headspace gas composition during microbiome-based medium-chain CA formation based on ethanol and acetate on three different scales.The proportion of H_2 permanently increased on all three scales, confirming the activity of the proposed reverse β-oxidation pathway on serum bottle (110 mL, n=3) **(A)**, lab flask (440 mL, n=3) **(B)**, and conventional bioreactor (2.2 L, n=3) **(C)** scale. Furthermore, no methane was detected, thus no carbon for medium-chain CA formation was lost in methane formation.

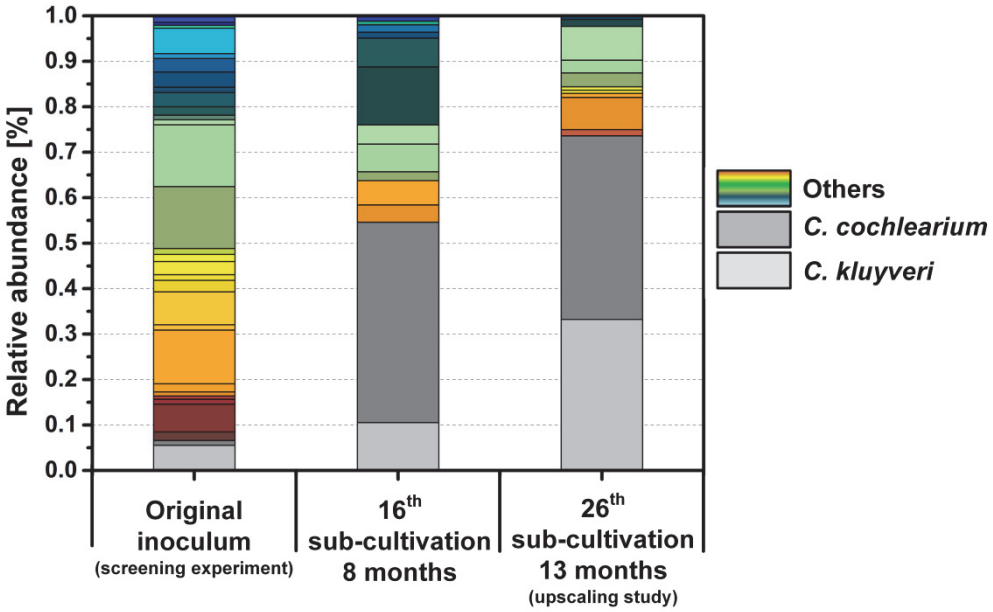

Figure S3: Evolution of community composition over time based on the DNA fingerprinting technique T-RFLP: The bars represent the major terminal restriction fragments (tRFs) after restriction digestion with HaeIII. The color code in the legend represents the different tRFs of a certain length with the corresponding tRFs of *C. kluyveri* and C. *cochlearium* in grey shades being identified by T-RFLP of the corresponding single clones after sequencing. The most abundant microorganisms after 16 and 26 sub-cultivation cycles were closest related to *Clostridium kluyveri* and *Clostridium cochlearium*.

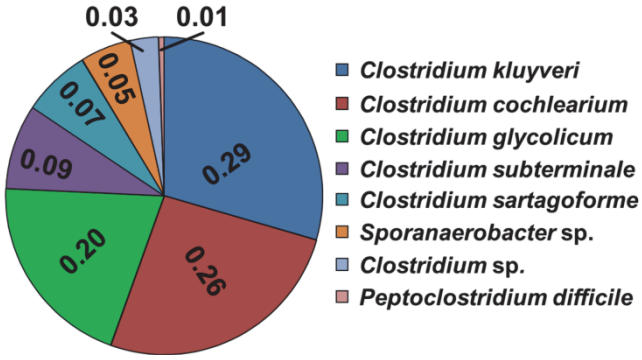

Figure S4: Community composition: a representative sample of a bioreactor (t = 0 h) was analyzed for its community composition based on cloning and sequencing of 167 clones. The microbiome was dominated by Clostridia being represented by four different families (RDP classifier: Clostridiaceae 1, Peptostreptococcaceae, Clostridiales_Incertae Sedis XI, Ruminococcaceae). The most abundant microorganisms were closest related to *Clostridium kluyveri*, *Clostridium cochlearium* and *Clostridium glycolicum* (highest BLAST score).

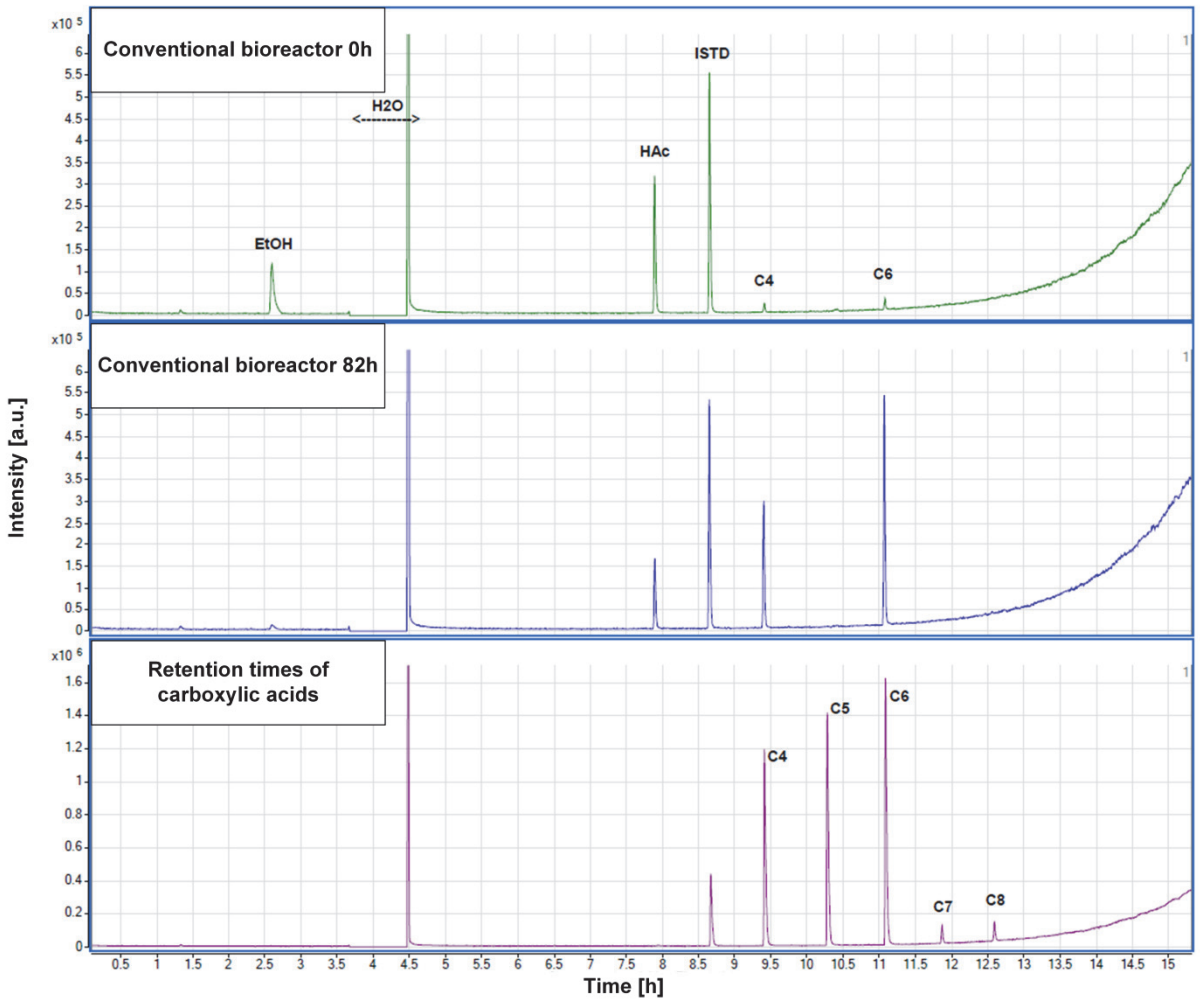

Figure S5: Representative GC-MS chromatograms for the conventional bioreactor after inoculation (0 h, top), at the end of the cultivation (82 h, middle) and for the retention times (bottom) of propionate (C_3, ISTD (internal standard), *n*-butyrate (C_4), *n*-valerate (C5), *n*-caproate (C_6), *n*-heptanoate (C_7) and *n*-caprylate (C_8). No peaks for propionate and *n*-valerate (C_5), *n*-heptanoate (C_7) and *n*-caprylate (C_8) were present in the bioreactor sample at the end of the cultivation (middle).