Carbon isotope fractionation during catabolism and anabolism in acetogenic bacteria growing on different substrates

- Supplementary

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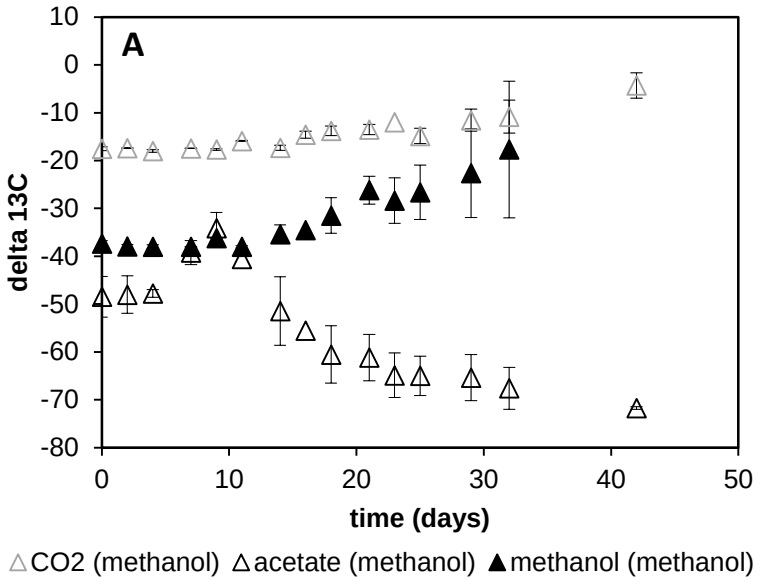
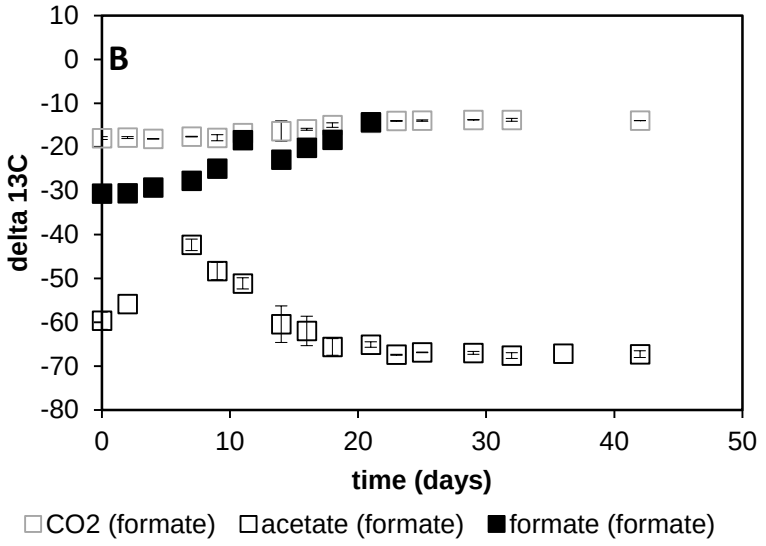
Table S1 Fractionation into biomass for different organisms

Organism	metabolism	substrate	temp	$\delta_{substrate}$	$\delta_{biomass}$	$\Delta_{substrate-biomass}$	Reference
Acetobacterium woodii	acetogen	CO2	28	-49.5	-76.3	-26.8	Preuss et al. 1989
Acetobacterium woodii	acetogen	CO2	28	-49.8	-70.4	-20.6	Preuss et al. 1989
Acetobacterim carbinolicum	acetogen	CO2	37	-9.7	-26.8	-17.1	unpublished *
Acetonema longum	acetogen	CO2	37	-10.1	-25.7	-15.6	unpublished *
Acetobacterium woodii	acetogen	CO2	28	-47.6	-62.4	-14.8	Preuss et al 1989
Thermoanaerobacter kivui	acetogen	CO2	55	-13.3	-27.9	-14.6	unpublished *
Thermoanaerobacter kivui	acetogen	CO2	55	-13.5	-26.9	-13.4	unpublished *
Moorella theromautotrophicum DSM 1974	acetogen	CO2	55	-13.4	-26.3	-12.9	unpublished *
Clostridium magnum	acetogen	CO2	37	-10.0	-22.7	-12.7	unpublished *
Clostridium aceticum	acetogen	CO2	37	-9.5	-21.7	-12.2	unpublished *
Acetitomaculum ruminis	acetogen	CO2	37	-10.0	-20.8	-10.8	unpublished *
Moorella thermoacetica DSM 2955	acetogen	CO2	55	-10.3	-20.0	-9.7	unpublished *
Sporomusa ovata	acetogen	CO2	37	-11.2	-20.9	-9.7	unpublished *
Moorella thermoacetica DSM 2955	acetogen	CO2	55	-12.9	-22.2	-9.3	unpublished *
Moorella thermoacetica DSM 2955	acetogen	CO2	55	-12.8	-21.7	-8.9	unpublished *
Acetobacterium woodii	acetogen	CO2	37	-11.8	-20.5	-8.7	unpublished *
Thermoanaerobacter kivui	acetogen	CO2	55	-10.7	-18.8	-8.1	unpublished *
Moorella theromautotrophicum DSM 1974	acetogen	CO2	55	-12.9	-19.2	-6.3	unpublished *
Thermoanaerobacter kivui	acetogen	glucose	55	-13.1	-21.5	-8.4	unpublished *
Thermoanaerobacter kivui	acetogen	glucose	55	-13.4	-18.7	-5.3	compare Fig. S4A
Thermoanaerobacter kivui	acetogen	glucose	55	-12.8	-15.2	-2.4	unpublished *
Moorella thermoacetica DSM 2955	acetogen	glucose	55	-13.4	-15.6	-2.2	unpublished *
Thermoanaerobacter kivui	acetogen	glucose	55	-26.2	-26.9	-0.8	unpublished *
Thermoanaerobacter kivui	acetogen	glucose	55	-13.0	-13.5	-0.5	unpublished *
Rhodococcus	fermentation	glucose				-2.3	Zyakun et al. 2013
E.coli	fermentation	glucose		-10.2	-11.9	-1.7	Zhang et al. 2002
Pseudomonas aureofaciens	fermentation	glucose				-1.0	Zyakun et al. 2013
E.coli	fermentation	glucose		-9.0	-9.6	-0.6	Zhang et al. 2002
E.coli	fermentation	glucose		-10.0	-9.7	0.3	Zhang et al. 2002
Pseudomonas aeruginosa	fermentation	glucose		-13.3	-10.3	3.0	Zhang et al. 2002
Methanosarcina barkeri	methanogen	acetate	37	-30.9	-38.2	-7.3	Londry et al. 2008
Methanosarcina barkeri	methanogen	acetate	37	-30.9	-31.4	-0.5	Londry et al. 2008
Methanosarcina barkeri	methanogen	acetate	37	-25.3	-19.1	6.2	Goevert et al. 2009
Methanosarcina acetivorans	methanogen	acetate	37	-25.6	-18.5	7.1	Goevert et al. 2009
M. lithoautotrophicus	methanogen	CO2	65	-23.2	-51.2	-28.0	House et al. 2003
M. lithoautotrophicus	methanogen	CO2	65	-21.5	-49.1	-27.6	House et al. 2003
M. lithoautotrophicus	methanogen	CO2	65	-23.3	-50.2	-26.9	House et al 2003
M. lithoautotrophicus	methanogen	CO2	65	-23.4	-49.4	-26.0	House et al. 2003
M. lithoautotrophicus	methanogen	CO2	70	-23.7	-49.4	-25.7	House et al. 2003
Methanosarcina barkeri	methanogen	CO2	37	-20.5	-44.5	-24.0	House et al. 2003
M. lithoautotrophicus	methanogen	CO2	65	-23.9	-47.6	-23.7	House et al. 2003
M. thermoautotrophicus	methanogen	CO2	45	-23.7	-46.4	-22.7	House et al. 2003
M. lithoautotrophicus	methanogen	CO2	60	-22.7	-44.9	-22.2	House et al. 2003
M. thermoautotrophicus	methanogen	CO2	65	-24.7	-46.7	-22.0	House et al. 2003
M. janaschii	methanogen	CO2	85	-26.0	-43.6	-17.6	House et al. 2003
M. thermoautotrophicus	methanogen	CO2	45	-21.9	-39.1	-17.2	House et al. 2003
Methanobacterium thermoautotrophicus	methanogen	CO2	65	-23.2	-40.2	-17.0	House et al. 2003
M. lithoautotrophicus	methanogen	CO2	51	-22.1	-38.9	-16.8	House et al. 2003
Methanosarcina barkeri	methanogen	CO2	37	-28.5	-44.1	-15.6	Londry et al. 2008
M. janaschii	methanogen	CO2	85	-25.0	-39.6	-14.6	House et al. 2003
Methanosarcina barkeri	methanogen	CO2	37	-31.2	-45.1	-13.9	Londry et al. 2008
Methanopyrus kandleri	methanogen	CO2	100	-24.3	-37.4	-13.1	House et al. 2003
M. janaschii	methanogen	CO2	85	-24.8	-36.7	-11.9	House et al. 2003
M. lithoautotrophicus	methanogen	CO2	41	-21.9	-33.3	-11.4	House et al. 2003
M. thermoautotrophicus	methanogen	CO2	65	-23.1	-34.4	-11.3	House et al. 2003
M. thermoautotrophicus	methanogen	CO2	45	-21.9	-30.8	-8.9	House et al. 2003
M. janaschii	methanogen	CO2	85	-23.5	-31.3	-7.8	House et al. 2003
Methanosarcina barkeri	methanogen	methanol	37	-46.2	-77.3	-31.1	Londry et al. 2008
Methanolobus zinderi	methanogen	methanol	37	-38.6	-62.4	-23.8	Penger et al. 2013
Methanosarcina acetivorans	methanogen	methanol	37	-39.0	-62.8	-23.8	Penger et al. 2013
Methanosarcina barkeri	methanogen	methanol	37	-38.4	-62.1	-23.7	Penger et al. 2013
Methanosarcina barkeri	methanogen	methanol	37	-46.2	-46.1	0.1	Londry et al. 2008
Ectothiorhodospira shaposhnikovii	phototroph	CO2				-15.0 to -34.3	Zyakun et al. 2009
Lamprocystis purpureus	phototroph	CO2				-10.4 to -31.9	Zyakun et al. 2009
Thiocapsa sp.	phototroph	CO2				-21.9 to -29.1	Zyakun et al. 2009
Prosthecochloris sp.	phototroph	CO2				-18.3 to -22.7	Zyakun et al. 2009
Desulfobacter hydrogenophilus	sulfate reducer	acetate	30	-25.3	-32.7	-7.4	Goevert et al. 2008
Desulfobacter postgatei	sulfate reducer	acetate	30	-25.6	-31.2	-5.6	Goevert et al. 2008
Desulfobacterium autotrophicum	sulfate reducer	CO2	28	-49.5	-85.4	-35.9	Preuss et al. 1989
Desulfobacterium autotrophicum	sulfate reducer	CO2	28	-49.8	-84.8	-35.0	Preuss et al. 1989
Desulfobacter hydrogenophilus	sulfate reducer	CO2	28	-47.6	-60.9	-13.3	Preuss et al. 1989
Desulfobacter hydrogenophilus	sulfate reducer	CO2	28	-49.8	-59.2	-9.4	Preuss et al. 1989
Desulfobacter hydrogenophilus	sulfate reducer	CO2	28	-49.5	-57.9	-8.4	Preuss et al. 1989

unpublished *: Most of the data shown for acetogenic pure cultures shown here orginates form a study published in Blaser et al. Appl.Environ.Microbiol (2013). In general the growth conditions were similar to the ones described in the Material and Methods section of current publication. Multipe entries for a given strain are for replicated incubations of the given strain.





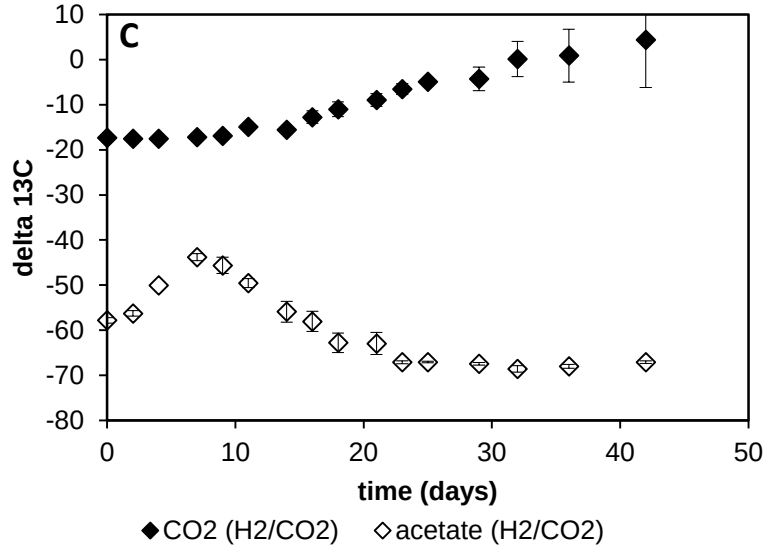


Figure S1 individual plots of the isotopic value of the data shown in Fig 2. S. *sphaeroides* grown on different substrates (A = methanol, B = formate, C = H_2/CO_2).

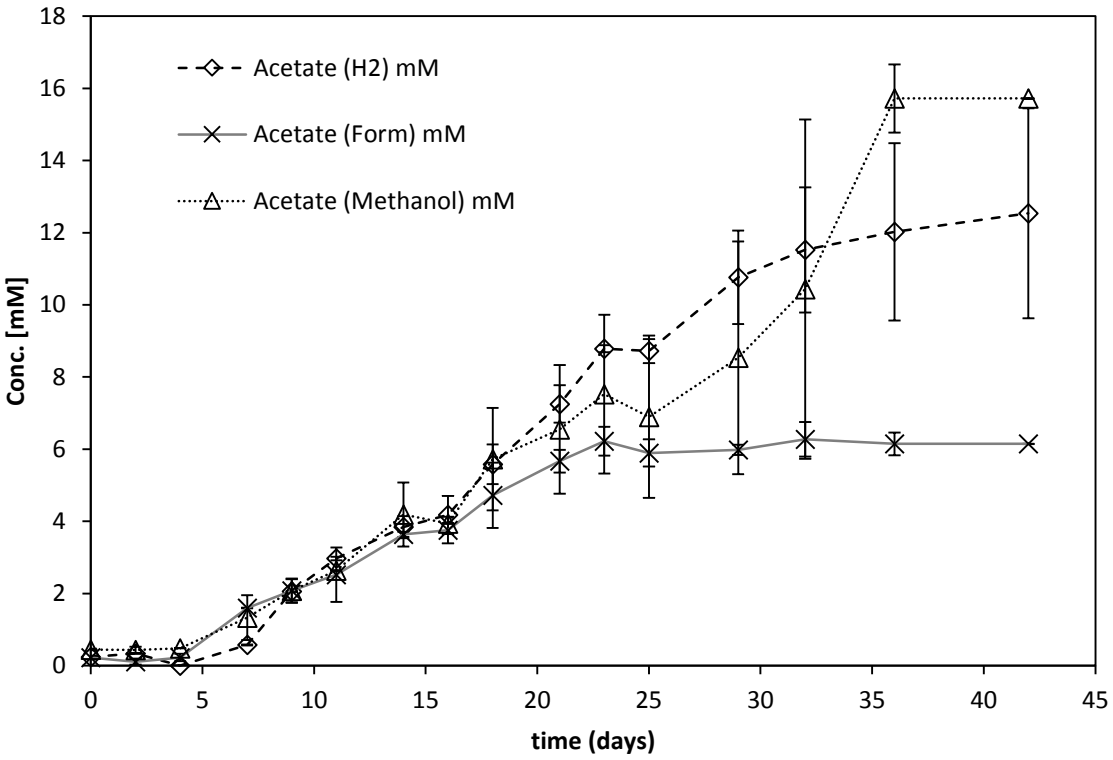


Figure S2 Acetate formation for *S. sphaeroides* grown on the expense of methanol (20mM) formate (20mM) or H_2/CO_2.

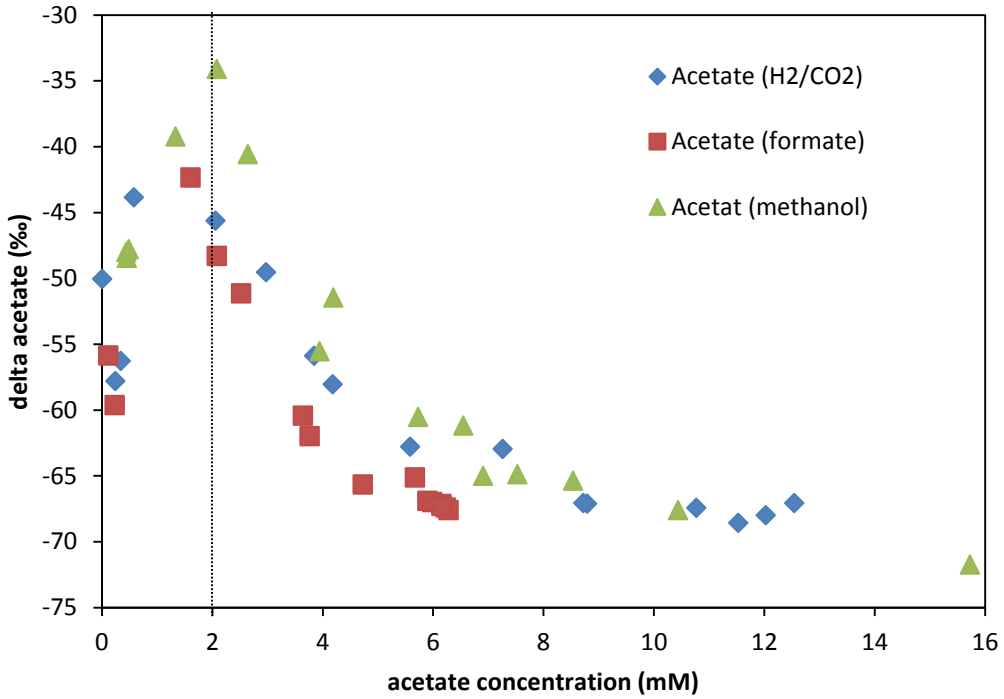


Figure S3 Acetate concentration vs. isotopic signal of acetate for *S. sphaeroides* grown on different substrates (compare Fig 2 for isotope values over time, Fig S2 for the individual isotope plots and Fig S3 for the acetate formation over time). While initially the delta ^{13}C value of the acetate increases (until about day 10; compare Fig. 2) it later decrease to reach -65 to -70‰.

Discussion:

Usually an increase in the delta ^{13}C of a compound origin in the consumption of this compound where the light isotopes are preferentially transferred to the product leaving the remaining substrate isotopically enriched. The depletion of the acetate delta ^{13}C value could therefore be caused by a depletion of the acetate pool by either reverse acetogenesis (which so far has only been described for syntrophic associations) or cell growth on the expense of acetate. However, as can be seen in Fig. S7 the acetate values are roughly constant during the first five days of incubation and then linearly increase with a similar slope for all tested substrates. Hence a depletion of the acetate pool seems unlikely.

Alternatively a depletion of the acetate signal can be explained by an exchange reaction of the carboxyl-group of acetate with the carbonate / bicarbonate pool (1-4). The acetate concentrations are very low at that time (below 2mM = 100µmol) while the total inorganic carbon pool (bicarbonate

buffer 4.5 mmol + CO2 in headspace 0.6 mmol) is relatively large. Such an exchange would therefore incorporate relatively heavy carbon (-17 to -18‰ for the headspace CO_2) with an isotopically more depleted carboxyl-group of the acetate and hence depleted the overall signal of the acetate while leaving the signal of the bicarbonate pool largely unchanged.

Similarly such an exchange reaction could help to explain the sharp decrease in the delta ^{13}C value of format in the *T. kivui* incubations under mixotrophic conditions shown in Fig 3 between day 14 and day 16. At that time the formate concentration is very low (compare Fig S5). Again the sharp decrease in formate can either be explain be newly formed formate which has been fractionated from e.g. CO_2; or it could originate from an exchange reaction of the formate pool with the very light acetate pool. Since the formate concentration in all other incubations (all of which) under H_2/CO_2 never drops to such low values (Fig. S5) a similar effect may be masked.

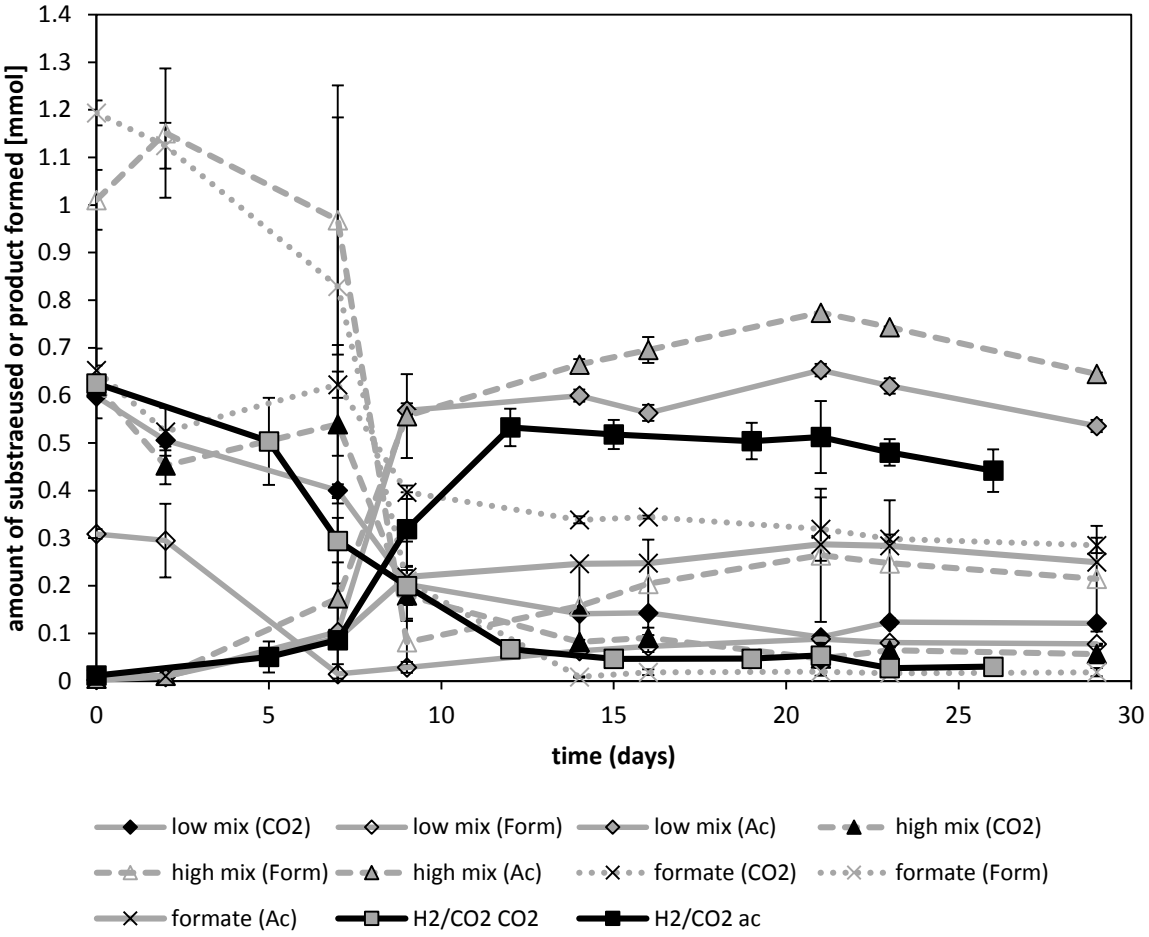


Figure S4 Substrate and product formation for *T.kivui* grown mixotrophic on different ratios of formate and H_2/CO_2.

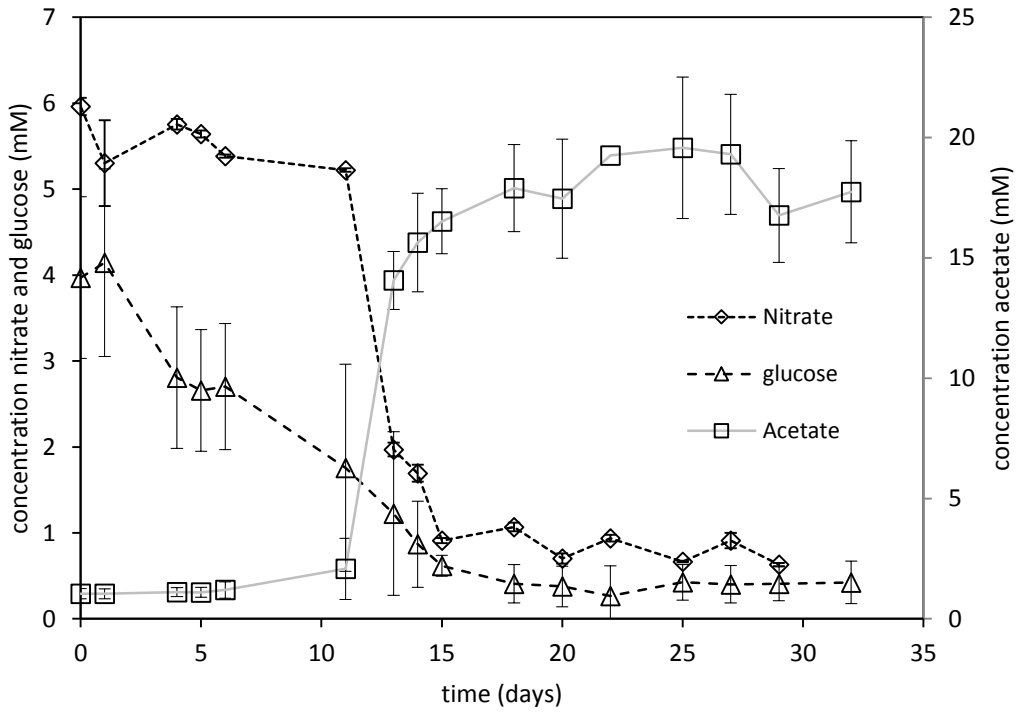


Figure S5. Concentration changes of substrate glucose, nitrate and the product acetate over time for *M. thermoacetica*. The concentration of nitrate was quantified using an HPLC-ion chromatography system. (n = 3; values given ± SD)

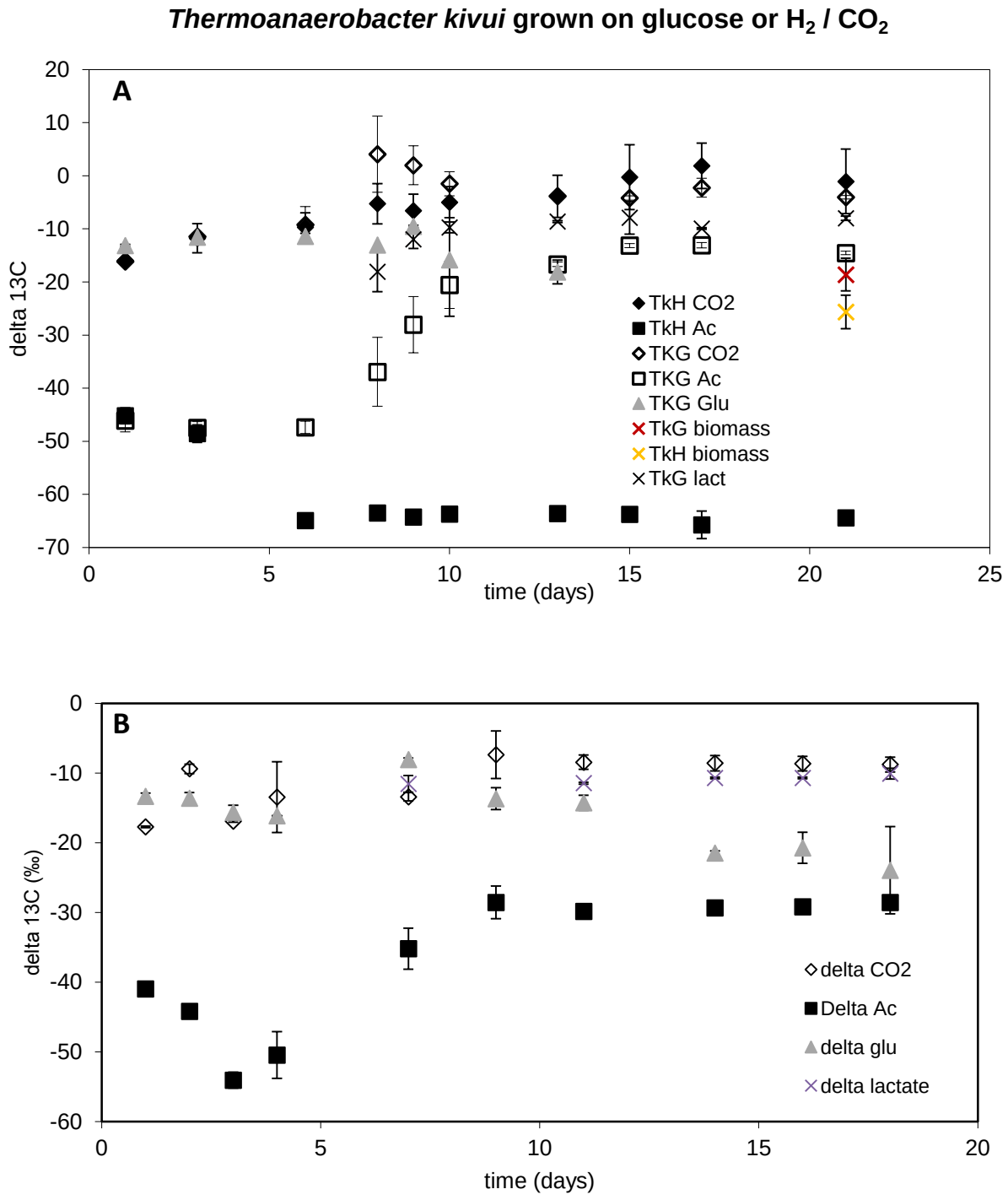


Figure S6. **A:** *T. kivui* grown on glucose TkG or H_2/CO_2 TkH. Depicted are the isotopic values of the glucose (Glu), CO_2, acetate and lactate (lact). The initial 52.4 mM glucose have been converted to 35.7 mM lactate and 81 mM acetate (89.7 % carbon recovery). The fractionation between CO_2 and acetate is $\varepsilon_{H2/CO2}$ = -53.0 ‰) the fractionation between glucose and lactate was ε_{Glu} = -14.1 ‰. **B:** *T.kivui* grown on glucose in a phosphate buffered medium (The medium composition is largely the same but containing a phosphate buffer instead of the bicarbonate; compare Blaser *et al.* 2015 (5)). The initial 9.7 mM glucose have been converted to 6.5mM lactate and 15.4 mM acetate (carbon recovery 86.6 %) The fractionation between glucose and acetate was ε_{Glu} = -13.4 ‰.

Discussion:

It should be noted that the experimental conditions in the experiments shown in Fig S6 are different from the conditions during the fractionation into the biomass experiment depicted in Fig. 4. In the later experimental set up a lower glucose concentration (4mM) was used; no lactate was formed. In contrast to all other experiments having an initial glucose delta ^{13}C value of about ε_{Glu} = -14 ‰ the initial glucose isotope signal in the biomass experiment (Fig. 4) was around ε_{Glu} = -27 ‰. This may by one explanation why the fractionation factor for glucose into biomass as well as into acetate is unexpected low in this experiment. Indeed we would have expected an intermediate fractionation factor of -15 to -20 ‰ like we observed in Fig. 3 & Fig. S6. While part of the electrons for the data in Fig. 6 are directed to lactate formation due to the huge substrate excess, we indeed would expect an even slightly lower fractionation in the experimental data shown in Fig. 4.

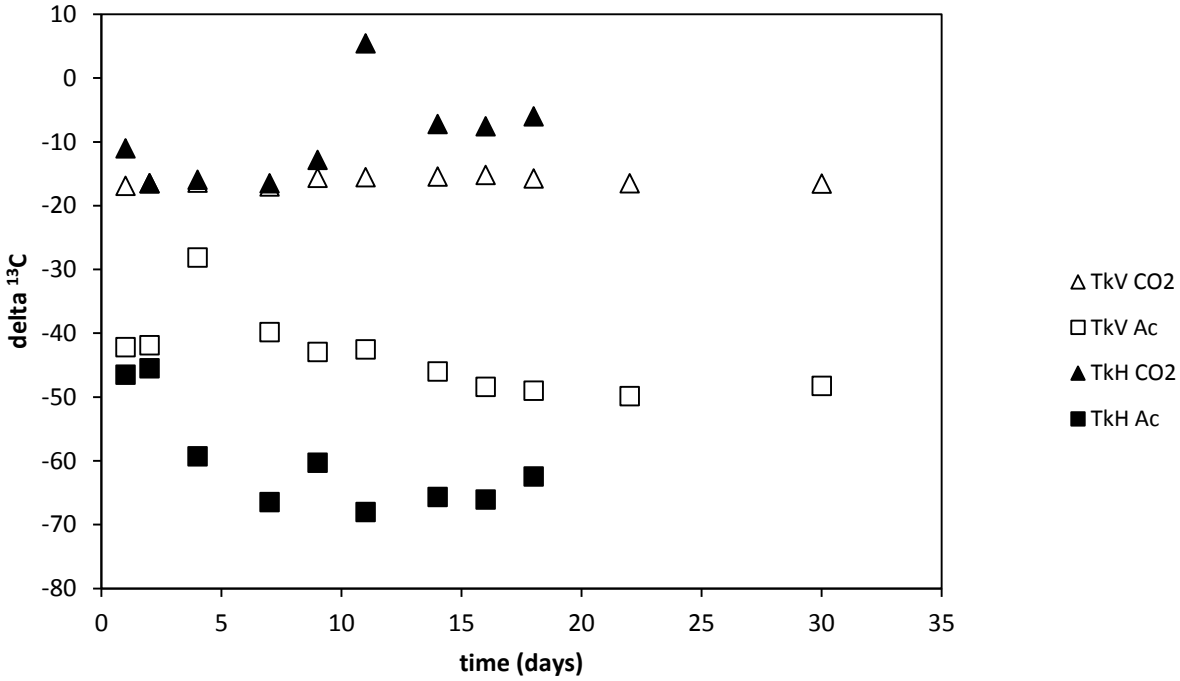


Figure S7. *Thermoanaerobacter kivui* growing on the expense of vanillic acid (TkV) or H_2/CO_2 (TkH). Shown is the isotopic signal of CO_2 (triangles) as well as acetate (squares). The isotopic signal of the vanillic acid could not be validated using our HPLC-IRMS approach. The initial $\delta^{13}C$ value of the vanillic acid was δ_{van} = -26.7 ‰.

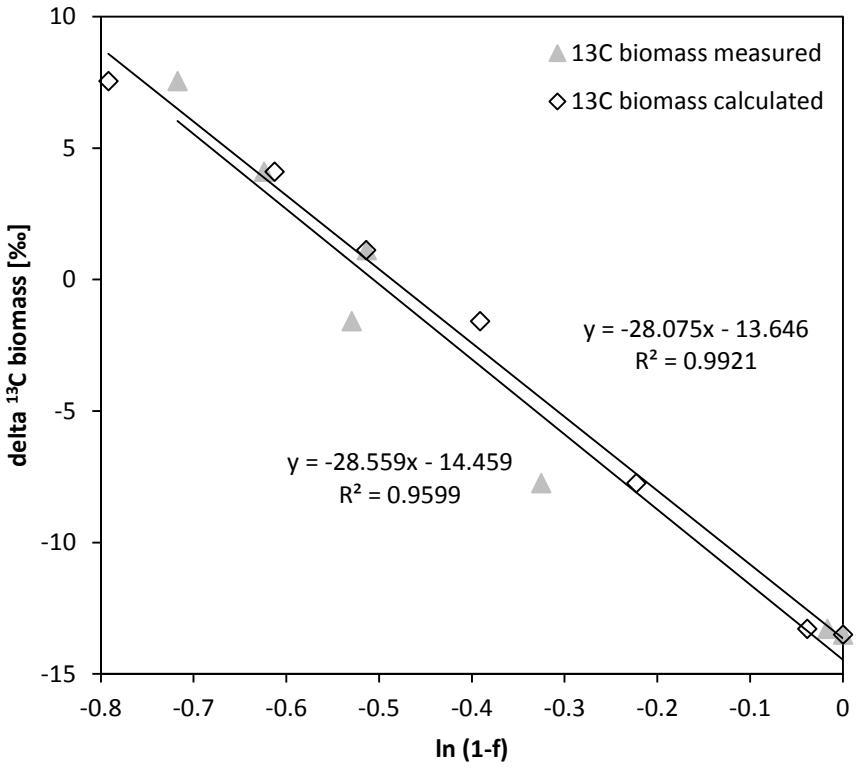


Figure S8. Comparison of the linear regression of the measured and calculated $\delta^{13}C$ values of the biomass. $\delta^{13}C$ values of the biomass were calculated assuming that the biomass is formed from pyruvate, which derives from carboxylation of acetate. Hence the $\delta^{13}C$ values of the biomass were calculated a simple mixing of the acetate signal (2/3) and the CO_2 of the headspace (1/3) for each measured time point. If the biomass would primarily steam directly from acetyl-CoA (e.g. fatty acids) the resulting isotopic signal would be expected to correspond to the original acetyl-CoA singal shown in Fig 4.

Discussion:

In order to generate bulk biomass, acetyl-CoA can either serve directly as building block to synthesize e.g. fatty acids or it can be carboxylated to pyruvate which than serves as the building block for e.g. amino acid synthesis. In the first case the isotopic signal of the biomass would resemble the delta ^{13}C depleted acetyl-CoA. In contrast carboxylation to pyruvate may incorporate heavier CO_2 resembling the delta ^{13}C value of the CO_2 in the headspace (or the total inorganic carbon) rendering an isotopic signal of the bulk biomass which is a mixed signal of the acetate (2/3) and CO_2 (1/3) ^{13}C values (**Fig. S8**). Assuming the second scenario a similar calculation can be performed for the data given in Londry *et al.* (6) for hydrogenotrophic methanogenesis yielding an isotope signal for the biomass of -44.6 ‰

(calculated) vs -45.1 ‰ (measured). It is however intriguing to note that the fractionation into biomass for *T. kivui* grown on glucose is likewise roughly 2/3 of the fractionation into acetate. However the absolute values ($\varepsilon_{catabol.}$ = + 4.2 ‰; $\varepsilon_{anabol.}$ = + 2.9 ‰) are quite similar and may not have any implications for environmental studies. Indeed one would assume that the biomass ($\delta_{biomass}$ = -27.3 ± 1.9 ‰) under these conditions steams from pyruvate formed during glycolysis and hence should have a similar isotope signal as the original glucose δ_{glu} = -27.9 ± 0.9‰.

SCFA determination using HPLC-IRMS

In previous publications we used a separate HPLC system operated under the similar conditions as the HPLC-IRMS to quantify the concentrations of SCFA and Glucose: We used the same eluent (1mM H_2SO_4 at 0.5ml/min) the same ion-exclusion column (Aminex HPX-87-H, BioRad, München, Germany) and run an UV-Detector (Sykam, Germany) as well as a Refraction Index detector. Data analysis was done using the Clarity Software package. This system provides a high sensitivity and due to the two detectors a high accuracy. The linear correlation usually covered the observed substrate / product concentrations of up to 20 mM and proved to have a high precision down to several µM.
For the isotope analysis the samples were usually diluted to have proximally 0.5 to 5 mM SCFA and analysed on the HPLC-IRMS system as described in the main manuscript. As can be exemplarily seen for acetate in Fig. S4 the HPLC-IRMS signal gives a linear response in this concentration range. Therefore, we directly used the HPLC-IRMS to quantify the concentration of SCFA in pure culture experiments. In this range the isotopic signal of acetate was -42.7 ± 0.3 ‰ irrespective of the acetate concentration. Repeated injections (n=10) of 1mM acetate gave an area of 397.8 ± 4.3 Vs. At higher concentrations the oxidation reaction of acetate to CO_2 is not complete, which is usually directly seen in the broadened peak shape of the Chromatogram.

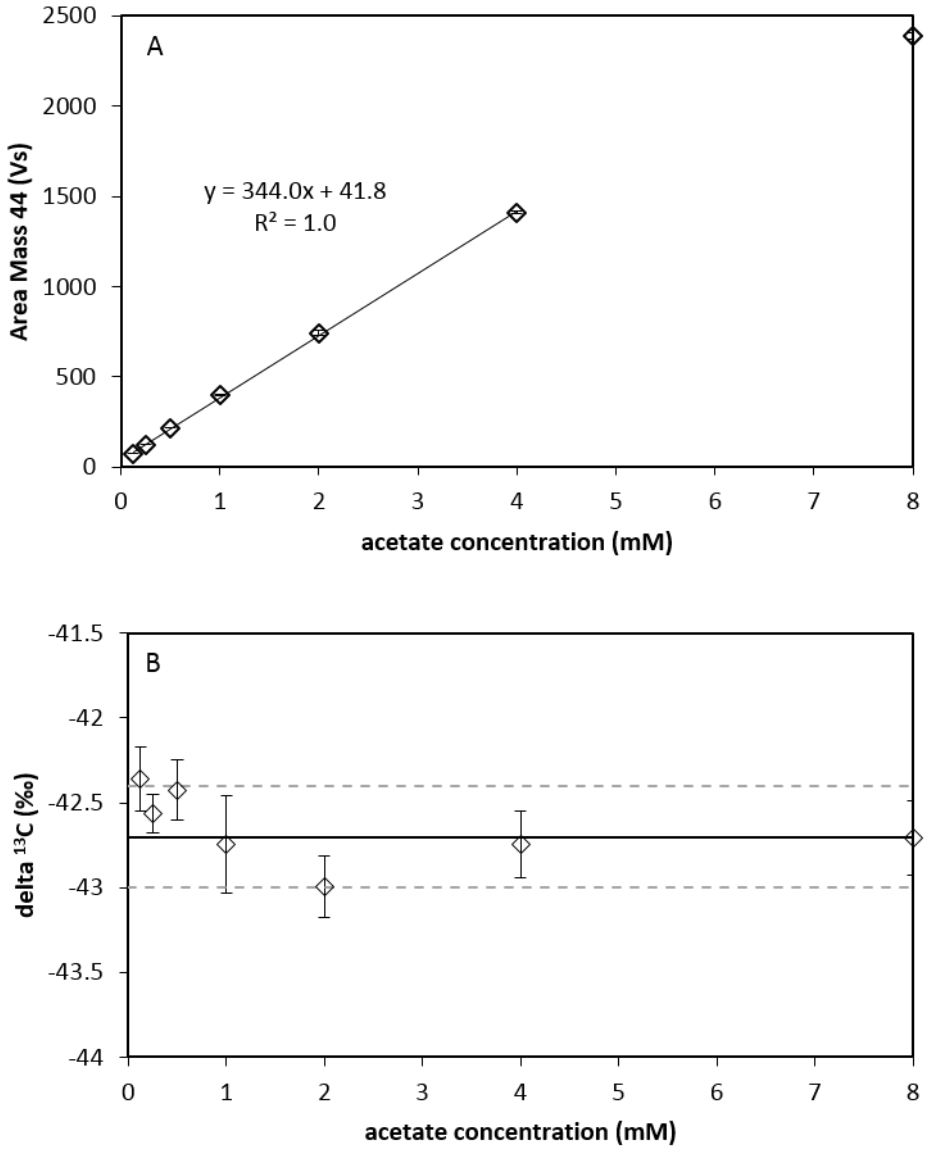


Figure S9 A) Acetate concentration determined by the HPLC-IRMS (n = 3 ± SD). **B)** isotopic signature of the respective acetate samples (n = 3 ± SD). The average of all samples (-42.7 ‰) is given as black line; the standard deviation (±0.7 ‰) as broken line.

Table S2 HPLC-IRMS results for different SCFA and glucose.

Analyte	Retention time (min)	Area 44 (Vs) of a 1mM solution	Delta 13C (‰)
Glucose	12.1	1287.8	-12.7
Formate	17.6	246.2	-24.8
Acetate	19.2	397.8	-42.7
Methanol	23.9	798.7	-29.8

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