# Electronic Supplementary Information

## S1. General values for all the cases studied

**Table S1:** Conditions (the same for all case studies)

| Variable | Value | Unit |
|---|---|---|
| $pH_{intracellular}$ | 7.00 | - |
| $pH_{extracellular}$ | 7.00 | - |
| T | 298.15 | K |
| $\Delta G_{ATP}$ | 50.00 | kJ/mol |
| $\Delta\Psi$ | -200.00 | mV |

**Table S2:** Standard energy for common reactions in all the pathways:

| Reaction | $\Delta G^0$ (kJ/mol) |
|---|---|
| $NADH + H^+ \rightarrow NAD^+ + H_2$ | -21.80 |
| $NADPH + H^+ \rightarrow NADP^+ + H_2$ | -25.50 |
| $2Fd_{red} + 2H^+ \rightarrow 2Fd_{ox} + H_2$ | -76.20 |
| $ADP + Pi + H^+ \rightarrow ATP + H_2O$ | -8.15 |

## S2. Quasi-equilibrium thermodynamic calculations

For each pathway the calculation of all the metabolites are carried out considering thermodynamic equilibrium ($\Delta G_{dis} = 0$) or including the energy dissipation referred in the subsequent tables (S4-S45). In the case that the reaction is coupled to a proton translocated across the membrane, the $\Delta\mu_{H+}$ energy is considered liberated in that reaction ($\Delta G_{dis} = \Delta\mu_{H^+}$) for calculation purposes.

$$\Delta G = \Delta G^0 + RTln \prod_i a_i \tag{1}$$

$$\Delta G = \Delta G_{dis} \rightarrow \prod_i a_i = e^{\left(\frac{\Delta G_{dis} - \Delta G^0}{RT}\right)} \tag{2}$$

We assume in all the cases that activities are equal to concentrations neglecting ionic strengths, assuming activity coefficients equal to 1.

The concentration of each conserved moiety in free form is calculated such that the total concentration of the conserved moiety in all its forms does not exceed the maximum concentration available in the cell. The calculation is iterative until the restriction is satisfied (see *Electronic Supplementary Information Excel example*).

As each pathway is a chain of series reactions, there it is always only one unknown, which can be solved knowing the energy dissipated by the reaction via Equation 1 (see ESI Excel example).

## S3. Assumptions and justification

### *S3.1. Total conserved moieties concentrations*

The values for the –CoA and –Pi total concentrations inside the cell are obtained from Kleerebezem and Stams, 2000[1] but –THF total concentration estimation was not found in any reference. Figure S1 illustrates the impact of different THF total concentration values within a reasonable range as not very significant for the purpose of this study.

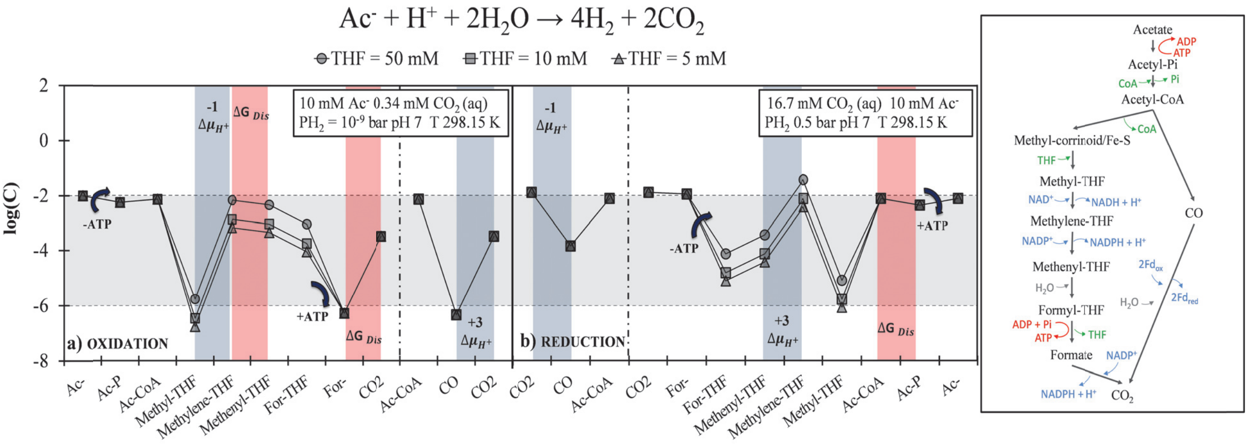

**Figure S1:** Limit (quasi equilibrium) metabolites concentrations (C, mol/L) for acetate oxidation (**a**) and $CO_2$ reduction (**b**) considering different total THF concentrations.

### *S3.2. Energy value of $\Delta\mu_{H+}$*

Proton motive force can be defined as the energy needed to translocate one proton across the cell membrane[2]. Its value is defined according to the chemiosmotic theory as in Equation 3.

$$\Delta\mu_{H+} = -F\Delta\Psi + RTln\, \frac{H^+_{out}}{H^+_{in}} \qquad (3)$$

In this study we use a constant electrical potential gradient ($\Delta\psi$) across the membrane of -200 mV and we assume no $\Delta pH$ considering in all the cases studied, a pH 7 inside and outside the cell compartment (Table S1). However, it is known that the membrane potential could vary depending on the conditions or species involved[3]. As well, some of the processes studied might be running under different pH, fact that might affect to the $\Delta\mu_{H+}$ value[4, 5].

In the processes running under energy scarcity conditions, the $\Delta\mu_{H+}$ value should be optimized with the homeostasis of the cell in order to survive. Indeed, if lower $\Delta\mu_{H+}$ values are considered, in some cases higher efficiency harvesting energy could be achieved allowing the cell to survive. An example on the effect of lowering $\Delta\mu_{H+}$ for acetate reduction to butyrate using ethanol as electron donor is presented (Figure S2).

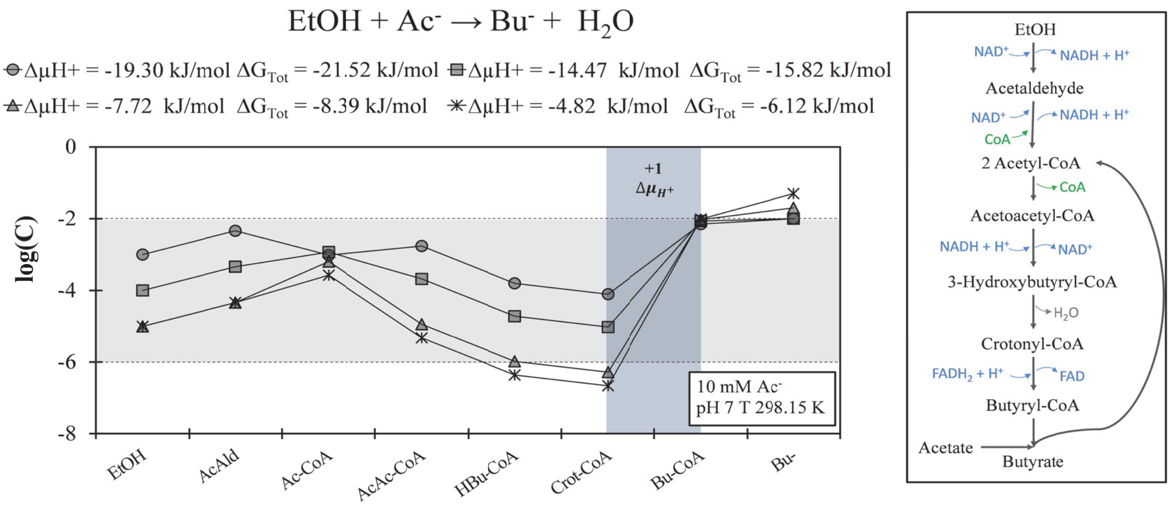

**Figure S2:** Limit (quasi equilibrium) metabolites concentrations (C, mol/L) for acetate reduction to butyrate using ethanol as electron donor considering different proton motive forces ($\Delta\mu_{H+}$). The $\Delta\mu_{H+}$ value optimization allows the cell to survive under lower energy available.

The $\Delta\mu_{H+}$ value optimisation could allow the cell to survive in lower energy available conditions like it is presented in Figure S2 but also it could increase the energy harvested for anabolism and maintenance as exposed in Figure S3.

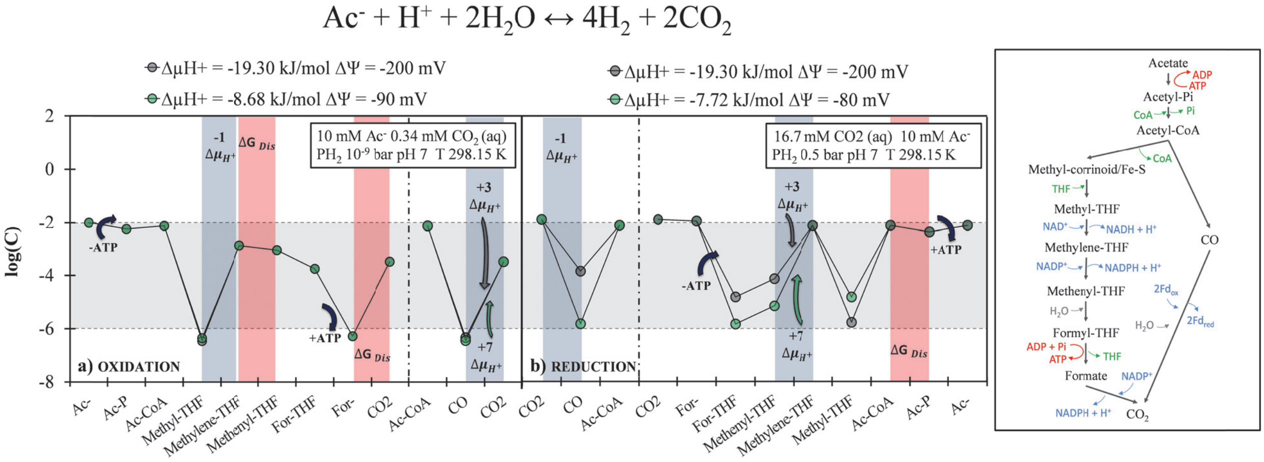

**Figure S3:** Limit (quasi equilibrium) metabolites concentrations (C, mol/L) for (**a**) acetate oxidation and for (**b**) $CO_2$ reduction considering optimised proton motive forces ($\Delta\mu_{H+}$) allowing the cell to harvest more energy per mol of product.

### *S3.3. External pH*

pH inside the cell is considered to be around 7 but outside it could be different. Changes in the external pH could increase or decrease the total energy available in the system to run a specific process but it has more impact in the intermediate metabolite concentrations due to the generation of a ΔpH gradient that affects the $\Delta\mu_{H+}$ value.

Agler *et al*[4] produced caproate with ethanol under pH 5.5. An analysis of the pathway considering external acidic pH demonstrates how the pathway keeps feasible even if the potential of the membrane does not change ($\Delta\psi$ = -200 mV) (Figure S4).

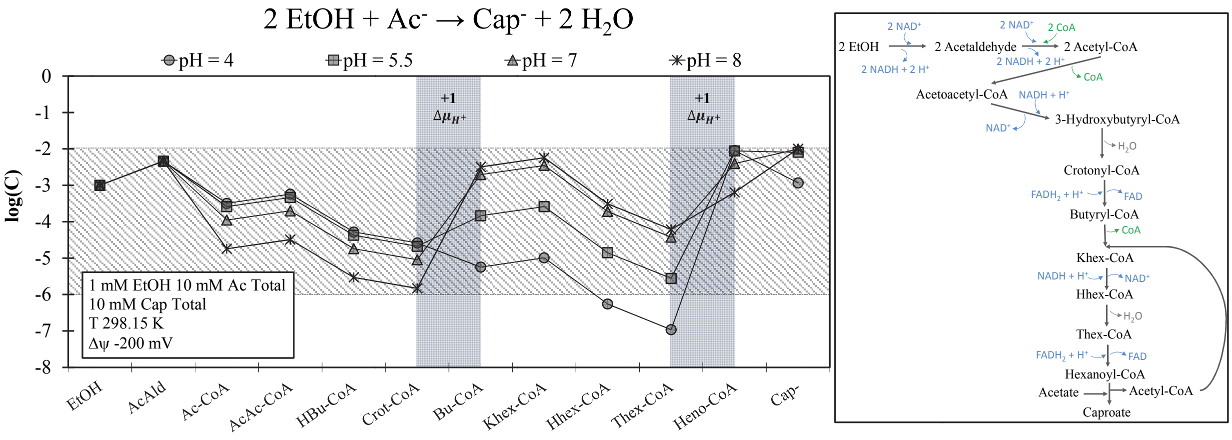

**Figure S4:** Limit (quasi equilibrium) metabolites concentrations (C, mol/L) for acetate reduction to caproate using ethanol as electron donor and considering different ΔpH

Nonetheless, as it was presented in Figure S2 and in Figure S3 the membrane potential could change, increasing the energy harvested to allow the cell to survive under energy scarcer conditions and reducing the minimum amount of energy needed to survive. Even in some cases, the change of the membrane potential could be a key factor to make a pathway feasible (Figure S5). Alcohol production runs experimentally under lower pH than 7[5] but when we analyze the pathway, a different potential gradient across the membrane than -200 mV seems to be needed in order to make the pathway feasible in acidic conditions (Figure S5).

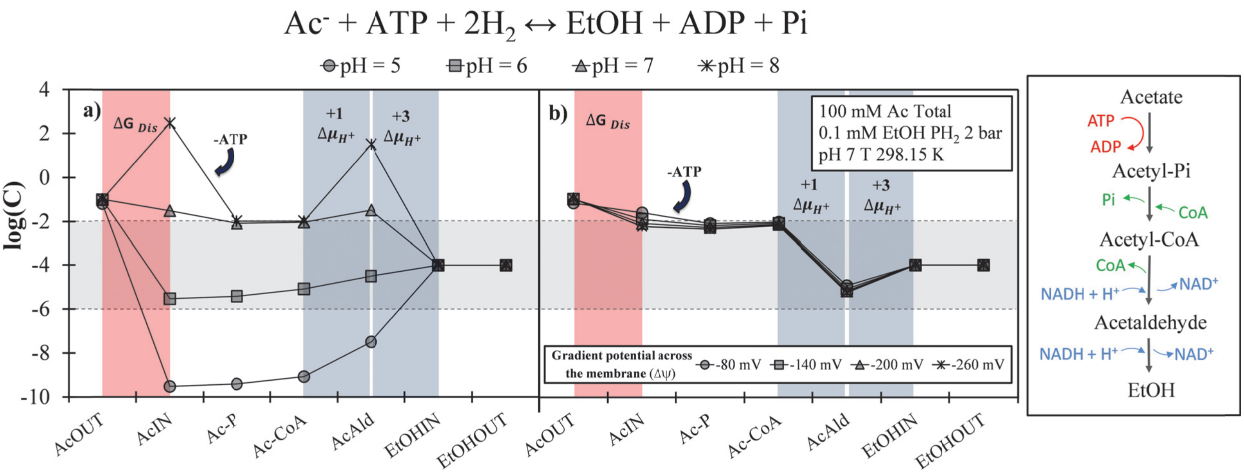

**Figure S5:** Limit (quasi equilibrium) metabolites concentrations (C, mol/L) for acetate reduction to ethanol considering different ΔpH at (**a**) the same membrane potential in all the cases and (**b**) with optimized membrane potential

## S4.Caproate production

Agler *et al*[4] reported butyrate elongation to caproate using ethanol. The oxidation of ethanol to acetate happens towards its later combination with butyrate for caproate production. Considering that all these metabolic reactions can occur in the same microorganism[6] we present in Figure S6 the analysis.

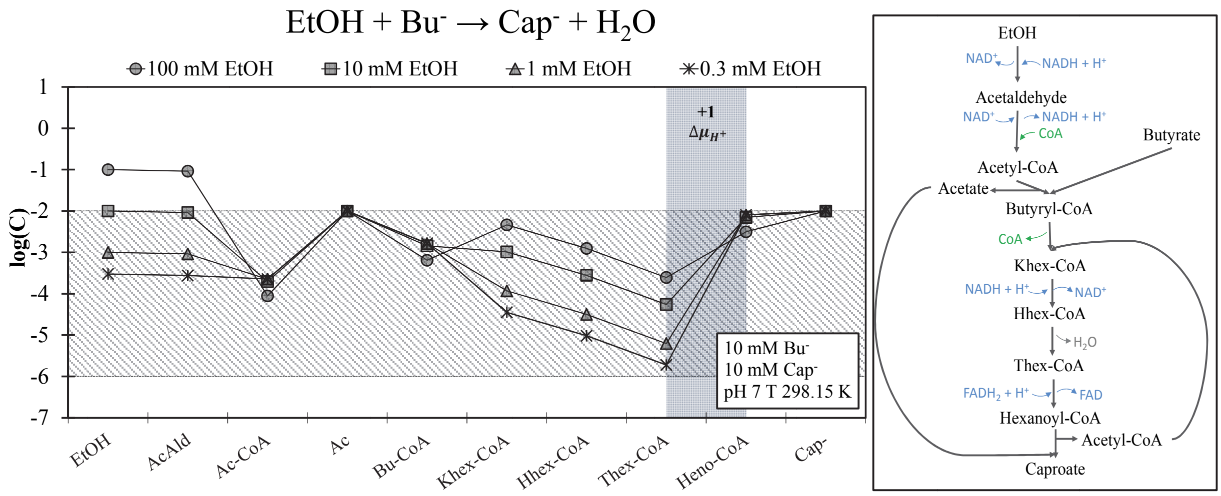

**Figure S6:** Limit (quasi equilibrium) metabolites concentrations (C, mol/L) for caproate production using ethanol and butyrate as substrates.

As well, the possibility of caproate production directly from ethanol as single substrate is analysed. In this case the process allows for more energy harvesting for growth and maintenance. The caproate production includes ethanol oxidation to acetic acid, which is later used in the butyrate elongation.

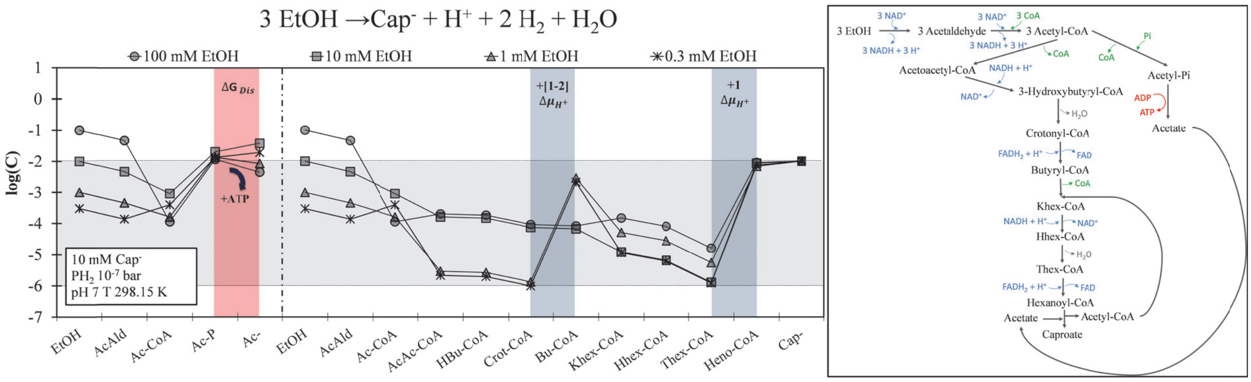

**Figure S7:** Limit (quasi equilibrium) metabolites concentrations (C, mol/L) for caproate production using only ethanol as substrate.

## S5. Alcohol production

Considering that reduction of the NAD$^+$ to recover NADH could be coupled with 2 proton translocations across the cell membrane, the pathway of ethanol production through acetate reduction seems feasible. The NADH recovery could occur with the electron transfer between a second electron carrier (e.g. NADPH) or with $H_2$ consumption. In this case, the total of the energy of 4 proton translocations could be harvested per mol of ethanol produced.

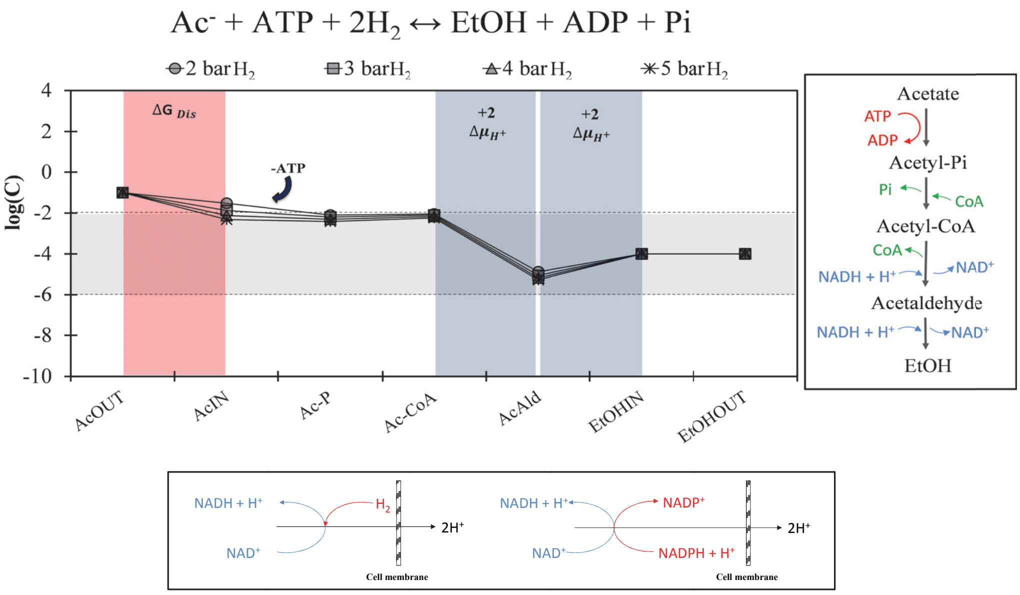

**Figure S8:** Limit (quasi equilibrium) metabolites concentrations (C, mol/L) for ethanol production with acetate reduction showing a feasible energy recovery alternative considering the NADH recovery coupled with two proton translocations across the membrane.

This option appears also feasible and does not imply that the Acetyl-CoA and Acetaldehyde reductions should have activity in the membrane. Moreover, proton translocations in electron changes between NAD$^+$ and NADPH were already pointed out as coupled with proton translocations in previous studies[7].

## S6. Acetate Oxidation/Reduction

**Table S3:** $\Delta G^0$ for acetate oxidation/reduction pathway.

| Reaction N° | $\Delta G^0$ (kJ/mol) |
|---|---|
| **Total** | 55.05 |
| **1** | -0.80 |
| **2** | 5.03 |
| **3+4** | 63.46[a] |
| **5** | 61.95 |
| **6** | 4.90 |
| **7** | 43.95 |
| **8** | 8.40 |
| **9** | -21.50 |
| **10** | 38.92 |

[a] Calculated considering the total $\Delta G^0$ of the pathway.

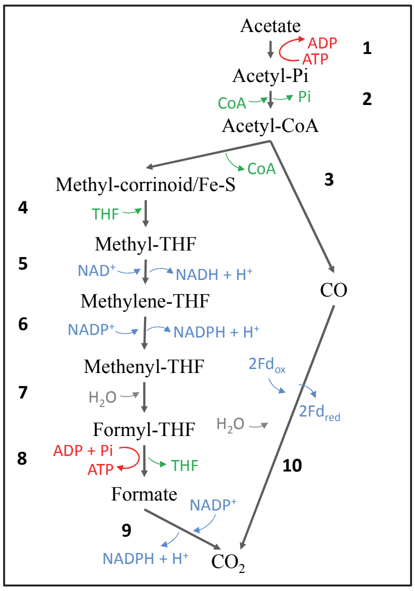

**Figure S7:** Acetate oxidation

*Data for Figure 1.a:*

**Table S4:** Acetate oxidation without energy dissipation and only $\Delta\mu_{H+}$ energy recovery at the CO oxidation site.

| $H_2$ | $\Delta G_{cat}$ | $Ac^-$ | Ac-P | Ac -CoA | Methyl -THF | Methylene -THF | Methenyl -THF | For -THF | $For^-$ | CO | $CO_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Bar** | **kJ/mol** | **mM** | | | | | | | | | |
| 1E-09 | -121.89 | 10.00 | 5.65 | 7.37 | 3.56E-14 | 3.29E-12 | 1.34E-06 | 2.66E-07 | 5.83E-10 | 5.98E+06 | 0.34 |
| 1E-06 | -53.40 | 10.00 | 5.65 | 7.37 | 3.56E-05 | 3.29E-06 | 1.34E-03 | 2.66E-04 | 5.83E-07 | 5.98E-03 | 0.34 |
| 1E-04 | -7.73 | 10.00 | 5.65 | 7.37 | 7.78 | 7.17E-03 | 2.92E-02 | 5.81E-03 | 5.83E-05 | 5.98E-09 | 0.34 |

*Data for Figure 1.b:*

**Table S5:** Acetate oxidation with energy dissipation and $\Delta\mu_{H+}$ energy recovery at the CO oxidation site and $\Delta\mu_{H+}$ energy invest at the methyl-THF oxidation site.

| $H_2$ | $\Delta G_{cat}$ | $Ac^-$ | Ac-P | Ac -CoA | Methyl -THF | Methylene -THF | Methenyl -THF | For -THF | $For^-$ | CO | $CO_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Bar** | **kJ/mol** | **mM** | | | | | | | | | |
| 1E-09 | -121.89 | 10.00 | 5.65 | 7.37 | 3.46E-04 | 1.36 | 0.91 | 1.82E-01 | 5.28E-04 | 4.65E-04 | 0.34 |
| 1E-06 | -53.40 | 10.00 | 5.65 | 7.37 | 1.09E-03 | 2.42E-01 | 0.52 | 1.03E-01 | 2.47E-04 | 1.79E-04 | 0.34 |
| 1E-04 | -7.73 | 10.00 | 5.65 | 7.37 | 1.45E-02 | 3.22E-02 | 1.31E-01 | 2.61E-02 | 5.83E-05 | 1.44E-05 | 0.34 |

**Table S6:** Energy dissipated and proton motive force invested or recovered proposed for anabolism and maintenance in the acetate oxidation pathway.

| $H_2$ | $\Delta G_{cat}$ | $\Delta G_{dis}$ in reac. 5 | $\Delta G_{dis}$ in reac. 6 | $\Delta G_{dis}$ in reac. 9 | $\Delta\mu_{H+}$[1] in reac. 5 | $\Delta\mu_{H+}$ in reac. 10 |
|---|---|---|---|---|---|---|
| **Bar** | **kJ/mol** | | | | — | |
| 1E-09 | -121.89 | -10 | -33 | -34 | -1 | +3 |
| 1E-06 | -53.40 | 0.00 | -13 | -15 | -1 | +2 |
| 1E-04 | -7.73 | 0.00 | 0.00 | 0.00 | -1 | +1 |

[1] Negative value implies energy investment in order to run the catabolic reactions. Positive value implies energy gained for anabolism.

*Data for Figure 2.a:*

**Table S7:** Acetate reduction without energy dissipation and only $\Delta\mu_{H+}$ energy recovery at the methylene-THF reduction site.

| $H_2$ | $\Delta G_{cat}$ | $CO_2$ | CO | $For^-$ | For -THF | Methenyl -THF | Methylene -THF | Methyl -THF | Ac -CoA | Ac-P | $Ac^-$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Bar** | **kJ/mol** | **mM** | | | | | | | | | |
| 0.5 | -96.11 | 16.78 | 7.35E-05 | 14.57 | 1.62E-03 | 8.12E-03 | 9.99 | 7.05E-10 | 7.37 | 5.65 | 10.00 |
| 1E-03 | -34.49 | 16.78 | 1.47E-06 | 0.29 | 7.72E-02 | 0.39 | 9.53 | 8.40E-05 | 7.37 | 5.65 | 10.00 |
| 1E-04 | -11.66 | 16.78 | 3.68E-08 | 7.28E-03 | 0.72 | 3.62 | 2.23 | 1.26 | 7.37 | 5.65 | 10.00 |

*Data for Figure 2.b:*

**Table S8:** Acetate reduction with energy dissipation and $\Delta\mu_{H+}$ energy recovery at the methylene-THF reduction site and $\Delta\mu_{H+}$ energy invest at the $CO_2$ reduction site.

| $H_2$ | $\Delta G_{cat}$ | $CO_2$ | CO | $For^-$ | For -THF | Methenyl -THF | Methylene -THF | Methyl -THF | Ac -CoA | Ac-P | $Ac^-$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Bar** | **kJ/mol** | **mM** | | | | | | | | | |
| 0.5 | -96.11 | 16.78 | 1.77E-01 | 14.57 | 1.80E-02 | 9.05E-02 | 9.89 | 1.88E-03 | 14.57 | 5.65 | 10.00 |
| 1E-03 | -34.49 | 16.78 | 3.53E-04 | 2.91E-02 | 5.22E-01 | 2.62 | 6.45 | 1.00E-02 | 9.99 | 5.65 | 10.00 |
| 1E-04 | -11.66 | 16.78 | 3.53E-05 | 2.91E-03 | 6.77E-01 | 3.40 | 0.84 | 3.07E-03 | 7.37 | 5.65 | 10.00 |

**Table S9:** Energy dissipated and proton motive force invested or recovered proposed for anabolism and maintenance in the acetate reduction pathway.

| $H_2$ | $\Delta G_{cat}$ | $\Delta G_{dis}$ in reac. 5 | $\Delta G_{dis}$ in reac. 2 | $\Delta\mu_{H+}$ in reac. 10 | $\Delta\mu_{H+}$ in reac. 5 |
|---|---|---|---|---|---|
| **Bar** | **kJ/mol** | | | — | |
| 0.5 | -96.11 | -6.00 | -50.00 | -1 | +3 |
| 1E-03 | -34.49 | 0.00 | -15.00 | -1 | +2 |
| 1E-04 | -11.66 | 0.00 | 0.00 | -1 | +1 |

*Data for Figure S1.a:*

**Table S10:** Acetate oxidation with different values of total THF. Energy dissipated and proton translocations are the same as the ones presented in Table S6 for the same conditions.

| THF | $H_2$ | $\Delta G_{cat}$ | $Ac^-$ | Ac-P | Ac -CoA | Methyl -THF | Methylene -THF | Methenyl -THF | For -THF | $For^-$ | CO | $CO_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **mM** | **Bar** | **kJ/mol** | **mM** | | | | | | | | | |
| 50 | 1E-09 | -121.89 | 10.00 | 5.65 | 7.37 | 1.73E-03 | 6.79 | 4.57 | 0.91 | 5.28E-04 | 4.65E-04 | 0.34 |
| 10 | 1E-09 | -121.89 | 10.00 | 5.65 | 7.37 | 3.46E-04 | 1.36 | 0.91 | 0.18 | 5.28E-04 | 4.65E-04 | 0.34 |
| 5 | 1E-09 | -121.89 | 10.00 | 5.65 | 7.37 | 1.73E-04 | 0.68 | 0.46 | 0.09 | 5.28E-04 | 4.65E-04 | 0.34 |

*Data for Figure S1.b:*

**Table S11:** Acetate reduction with different values of total THF. Energy dissipated and proton translocations are the same as the ones presented in Table S9 for the same conditions.

| THF | $H_2$ | $\Delta G_{cat}$ | $CO_2$ | CO | $For^-$ | For -THF | Methenyl -THF | Methylene -THF | Methyl -THF | Ac -CoA | Ac-P | $Ac^-$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **mM** | **Bar** | **kJ/mol** | **mM** | | | | | | | | | |
| 50 | 0.5 | -96.11 | 16.78 | 0.18 | 14.57 | 9.01E-02 | 0.45 | 49.45 | 9.40E-03 | 10.00 | 5.65 | 10.00 |
| 10 | 0.5 | -96.11 | 16.78 | 0.18 | 14.57 | 1.80E-02 | 9.05E-02 | 9.89 | 1.88E-03 | 10.00 | 5.65 | 10.00 |
| 5 | 0.5 | -96.11 | 16.78 | 0.18 | 14.57 | 9.01E-03 | 4.52E-02 | 4.94 | 9.40E-04 | 10.00 | 5.65 | 10.00 |

*Data for Figure S3.a:*

**Table S12:** Acetate oxidation with different values of $\Delta\mu_{H^+}$. Energy dissipated and proton translocations are the same as the ones presented in Table S6 for the same conditions.

| $\Delta\psi$ | $H_2$ | $\Delta G_{cat}$ | $Ac^-$ | Ac-P | Ac -CoA | Methyl -THF | Methylene -THF | Methenyl -THF | For -THF | $For^-$ | CO | $CO_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **mV** | **Bar** | **kJ/mol** | **mM** | | | | | | | | | |
| -200 | 1E-09 | -121.89 | 10.00 | 5.65 | 7.37 | 3.46E-04 | 1.36 | 0.91 | 0.18 | 5.28E-04 | 4.65E-04 | 0.34 |
| -90 | 1E-09 | -121.89 | 10.00 | 5.65 | 7.37 | 4.43E-04 | 1.36 | 0.91 | 0.18 | 5.28E-04 | 3.63E-04 | 0.34 |

*Data for Figure S3.b:*

**Table S13:** Acetate oxidation with different values of $\Delta\mu_{H^+}$. Energy dissipated and proton translocations are the same as the ones presented in Table S9 for the same conditions.

| $\Delta\psi$ | $H_2$ | $\Delta G_{cat}$ | $CO_2$ | CO | $For^-$ | For -THF | Methenyl -THF | Methylene -THF | Methyl -THF | Ac -CoA | Ac-P | $Ac^-$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **mV** | **Bar** | **kJ/mol** | **mM** | | | | | | | | | |
| -200 | 0.5 | -96.11 | 16.78 | 0.18 | 14.57 | 1.80E-02 | 9.05E-02 | 9.89 | 1.88E-03 | 10.00 | 5.65 | 10.00 |
| -80 | 0.5 | -96.11 | 16.78 | 1.65E-03 | 14.57 | 1.62E-03 | 8.11E-03 | 9.97 | 1.80E-02 | 10.00 | 5.65 | 10.00 |

## S7. Butyrate Oxidation/Acetate Reduction

**Table S14:** $\Delta G^0$ for butyrate oxidation/reduction pathway.

| **Reaction Nº** | **$\Delta G^0$ (kJ/mol)** |
|---|---|
| **Total** | -80.02 |
| **1** | -0.8 |
| **2** | 5.03 |
| **3** | 27.1 |
| **4** | -61.53 |
| **5** | 1.72 |
| **6** | -115.7 |
| **7** | 4.93 |

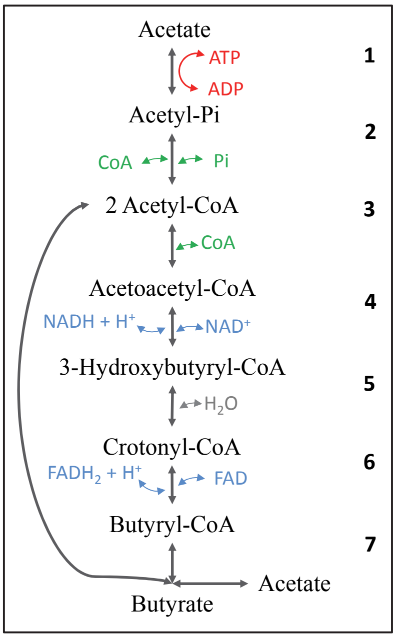

**Figure S8:** Acetate reduction to butyrate

*Data for Figure 3.a:*

**Table S15:** Butyrate oxidation pathway.

| $H_2$ | $\Delta G_{cat}$[a] | $Bu^-$ | Bu -CoA | Crot -CoA | HBu -CoA | AcAcetyl -CoA | Acetyl -CoA | Acetyl -Pi | $Ac^-$ |
|---|---|---|---|---|---|---|---|---|---|
| **Bar** | **kJ/mol** | **mM** | | | | | | | |
| 1E-07 | -4.52 | 10.00 | 1.28E-03 | 1.41E-03 | 2.83E-03 | 3.10E-03 | 2.18 | 1.15 | 1.00 |
| 5E-07 | 3.46 | 10.00 | 1.43E-02 | 3.15E-03 | 6.31E-03 | 1.38E-03 | 2.18 | 1.15 | 1.00 |
| 1E-06 | 6.89 | 10.00 | 3.80E-02 | 4.20E-03 | 8.40E-03 | 9.20E-04 | 2.18 | 1.15 | 1.00 |
| 1E-05 | 18.30 | 10.00 | 4.49E-02 | 4.97E-04 | 9.94E-04 | 1.09E-05 | 2.18 | 1.15 | 1.00 |
| 1E-04 | 29.72 | 10.00 | 3.11 | 3.43E-03 | 6.87E-03 | 7.52E-06 | 1.50 | 1.15 | 1.00 |

[a] Global Gibbs energy considering the reaction ($Bu^- + 2H_2O \rightarrow 2Ac^- + 2H_2 + H^+$). 2/3 of the 50 kJ/mol extra gained with the ATP production are invested in the catabolic pathway.

**Table S16:** Energy dissipated and proton motive force invested or recovered proposed for anabolism and maintenance in the butyrate oxidation pathway.

| $H_2$ | $\Delta G_{cat}$ | $\Delta G_{dis}$ in reac. 4 | $\Delta \mu_{H+}$ in reac. 5 |
|---|---|---|---|
| **Bar** | **kJ/mol** | | — |
| 1E-07 | -4.52 | -14 | -2 |
| 5E-07 | 3.46 | -12 | -2 |
| 1E-06 | 6.89 | -11 | -2 |
| 1E-05 | 18.30 | 0 | -2 |
| 1E-04 | 29.72 | 0 | -2 |

*Data for Figure 3.b:*

**Table S17:** Acetate reduction pathway.

| $H_2$ | $\Delta G_{cat}$[a] | $Ac^-$ | Acetyl -Pi | Acetyl -CoA | AcAcetyl -CoA | HBu -CoA | Crot -CoA | Bu -CoA | $Bu^-$ |
|---|---|---|---|---|---|---|---|---|---|
| **Bar** | **kJ/mol** | **mM** | | | | | | | |
| 1 | -42.51 | 10.00 | 5.65 | 1.46E-02 | 7.28E-07 | 6.65 | 3.32 | 1.06E-02 | 1.00 |
| 0.5 | -39.07 | 10.00 | 5.65 | 2.90E-02 | 1.45E-06 | 6.63 | 3.31 | 2.12E-02 | 1.00 |
| 0.1 | -31.09 | 10.00 | 5.65 | 0.14 | 7.08E-06 | 6.47 | 3.23 | 0.10 | 1.00 |
| 1E-03 | -8.26 | 10.00 | 5.65 | 3.61 | 1.80E-04 | 1.65 | 0.82 | 2.63 | 1.00 |
| 1E-05 | 14.43 | 10.00 | 5.65 | 4.77 | 2.39E-04 | 2.18E-02 | 1.09E-02 | 3.49 | 1.00 |

[a] Global Gibbs energy considering the reaction ($2Ac^- + 2H_2 + H^+ \rightarrow Bu^- + 2H_2O$). 50 kJ/mol extra are invested in the catabolic process with the hydrolysis of one ATP.

**Table S18:** Energy dissipated and proton motive force invested or recovered proposed for anabolism and maintenance in the acetate reduction pathway.

| $H_2$ | $\Delta G_{cat}$ | $\Delta\mu_{H+}$ in reac. 5 |
|---|---|---|
| **Bar** | **kJ/mol** | **–** |
| 1 | -92.51 | +4 |
| 0.5 | -89.07 | +4 |
| 0.1 | -81.09 | +4 |
| 1E-03 | -58.26 | +3 |
| 1E-05 | -35.43 | +1 |

## S8. Acetate reduction with ethanol and lactate

**Table S19:** $\Delta G^0$ for acetate reduction pathway with ethanol and lactate.

| Reaction Nº | $\Delta G^0$ (kJ/mol) |
|---|---|
| **Total for EtOH** | -38.65 |
| **Total for $Lac^-$** | -96.95 |
| **1** | 63.68 |
| **2** | 65.01 |
| **3** | 25.58 |
| **4** | 20.32 |
| **5** | 27.1 |
| **6** | -61.53 |
| **7** | 1.72 |
| **8** | -115.7 |
| **9** | 4.93 |

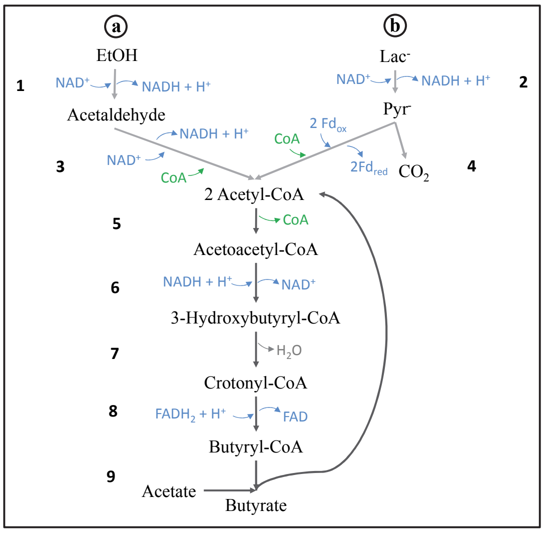

Figure S9: **Acetate reduction with ethanol and lactate**

*Data for Figure 4.a:*

**Table S20:** Acetate reduction pathway with ethanol to butyrate.

| EtOH | $\Delta G_{cat}$ | AcAld | Acetyl -CoA | AcAcetyl -CoA | HBu -CoA | Crot -CoA | Bu -CoA | $Bu^-$ | $Ac^-$ |
|---|---|---|---|---|---|---|---|---|---|
| **mM** | **kJ/mol** | **mM** | | | | | | | |
| 100.00 | -32.94 | 459.76 | 4.73E-02 | 8.45 | 0.77 | 0.39 | 0.35 | 10.00 | 10.00 |
| 10.00 | -27.23 | 45.98 | 0.35 | 6.24 | 0.57 | 0.29 | 2.55 | 10.00 | 10.00 |
| 1.00 | -21.53 | 4.60 | 0.97 | 1.73 | 0.16 | 7.89E-02 | 7.07 | 10.00 | 10.00 |
| 0.30 | -18.54 | 1.38 | 1.12 | 0.60 | 5.50E-02 | 2.75E-02 | 8.19 | 10.00 | 10.00 |

**Table S21:** Proton motive force recovered proposed for anabolism and maintenance in the acetate reduction pathway with ethanol.

| EtOH | $\Delta G_{cat}$ | $\Delta\mu_{H+}$ in reac. 8 |
|---|---|---|
| **mM** | **kJ/mol** | **—** |
| 100.00 | -32.94 | +1 |
| 10.00 | -27.23 | +1 |
| 1.00 | -21.53 | +1 |
| 0.30 | -18.54 | +1 |

*Data for Figure 4.b:*

**Table S22:** Acetate reduction pathway with lactate to butyrate.

| $Lac^-$ | $\Delta G_{cat}$ | $Pyr^-$ | Acetyl -CoA | AcAcetyl -CoA | HBu -CoA | Crot -CoA | Bu -CoA | $Bu^-$ | $Ac^-$ | $CO_2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **mM** | **kJ/mol** | **mM** | | | | | | | | |
| 100.00 | -41.59 | 0.27 | 2.03 | 0.60 | 55.02 | 27.49 | 14.85 | 10.00 | 10.00 | 1000.00 |
| 10.00 | -35.88 | 0.27 | 1.93 | 5.71 | 52.20 | 26.08 | 14.09 | 10.00 | 10.00 | 1000.00 |
| 1.00 | -30.17 | 0.27 | 1.27 | 37.73 | 34.47 | 17.22 | 9.30 | 10.00 | 10.00 | 1000.00 |
| 0.30 | -27.19 | 8.07E-02 | 3.40 | 30.26 | 27.65 | 13.81 | 24.87 | 10.00 | 10.00 | 1000.00 |

**Table S23:** Proton motive force recovered proposed for anabolism and maintenance in the acetate reduction pathway with lactate.

| $Lac^-$ | $\Delta G_{cat}$ | $\Delta\mu_{H+}$ in reac. 8 |
|---|---|---|
| **mM** | **kJ/mol** | **—** |
| 100.00 | -41.59 | +2 |
| 10.00 | -35.88 | +2 |
| 1.00 | -30.17 | +2 |
| 0.30 | -27.19 | +1 |

*Data for Figure S2:*

**Table S24:** Acetate reduction pathway with ethanol to butyrate. One $\Delta\mu_{H+}$ is harvested in reaction 8 for all the cases.

| EtOH | $\Delta G_{cat}$ | AcAld | Acetyl -CoA | AcAcetyl -CoA | HBu -CoA | Crot -CoA | Bu -CoA | $Bu^-$ | $Ac^-$ |
|---|---|---|---|---|---|---|---|---|---|
| **mM** | **kJ/mol** | **mM** | | | | | | | |
| 100.00 | -32.94 | 4.60 | 0.97 | 1.73 | 0.16 | 7.89E-02 | 7.07 | 10.00 | 10.00 |
| 10.00 | -27.23 | 0.46 | 1.18 | 0.21 | 1.92E-02 | 9.59E-03 | 8.59 | 10.00 | 10.00 |
| 1.00 | -21.53 | 4.60E-02 | 0.64 | 1.14E-02 | 1.04E-03 | 5.22E-04 | 9.35 | 20.00 | 10.00 |
| 0.30 | -18.54 | 4.60E-02 | 0.27 | 4.76E-03 | 4.35E-04 | 2.17E-04 | 9.73 | 50.00 | 10.00 |

## S9. Acetate reduction until Caproate with ethanol

**Table S25:** $\Delta G^0$ for acetate reduction pathway until caproate with ethanol.

| Reaction Nº | $\Delta G^0$ (kJ/mol) |
|---|---|
| **Total** | -77.41 |
| **1** | 63.68 |
| **2** | 25.58 |
| **3** | 27.1 |
| **4** | -61.53 |
| **5** | 1.72 |
| **6** | -115.7 |
| **7** | 27.1 |
| **8** | -60.23 |
| **9** | 4.02 |
| **10** | -118.4 |
| **11** | 8.85 |

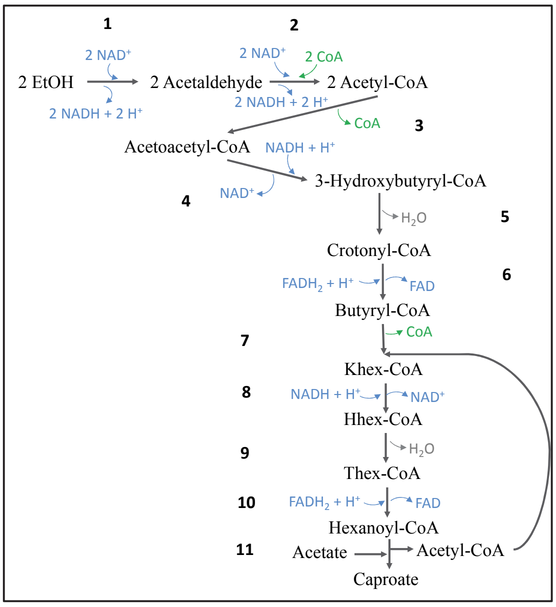

**Figure S10:** Acetate reduction until caproate

*Data for Figure 5:*

**Table S26:** Acetate reduction pathway with ethanol until caproate.

| EtOH | $\Delta G_{cat}$ | AcAld | Acetyl -CoA | AcAcetyl -CoA | HBu -CoA | Crot -CoA | Bu -CoA | Khex -CoA | Hhex -CoA | Thex -CoA | Heno -CoA | $Cap^-$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **mM** | **kJ/mol** | **mM** | | | | | | | | | | |
| 100.00 | -65.99 | 459.76 | 0.03 | 4.69 | 0.43 | 0.21 | 1.94E-02 | 3.46 | 0.19 | 3.70E-02 | 0.93 | 10.00 |
| 10.00 | -54.58 | 45.98 | 2.77E-03 | 4.95E-02 | 4.52E-03 | 2.26E-03 | 0.49 | 8.78 | 0.47 | 9.38E-02 | 0.10 | 10.00 |
| 1.00 | -43.16 | 4.60 | 0.11 | 0.20 | 0.02 | 9.06E-03 | 1.97 | 3.52 | 0.19 | 3.76E-02 | 3.94 | 10.00 |
| 0.30 | -37.19 | 1.38 | 0.22 | 0.12 | 0.01 | 5.38E-03 | 1.17 | 0.63 | 3.39E-02 | 6.71E-03 | 7.81 | 10.00 |

**Table S27:** Proton motive force recovered proposed for anabolism and maintenance in the acetate reduction pathway with ethanol until caproate.

| EtOH | $\Delta G_{cat}$ | $\Delta\mu_{H+}$ in reac. 6 | $\Delta\mu_{H+}$ in reac. 10 |
|---|---|---|---|
| **mM** | **kJ/mol** | — | |
| 100.00 | -65.99 | +2 | +1 |
| 10.00 | -54.58 | +1 | +1 |
| 1.00 | -43.16 | +1 | +1 |
| 0.30 | -37.19 | +1 | +1 |

*Data for Figure S4:*

**Table S28:** Acetate reduction pathway with ethanol until caproate with different external pH. One $\Delta\mu_{H+}$ is harvested in reaction 5 and another in reaction 9 for all the cases.

| pH | EtOH | AcAld | Acetyl-CoA | AcAcetyl-CoA | HBu-CoA | Crot-CoA | Bu-CoA | Khex-CoA | Hhex-CoA | Thex-CoA | Heno-CoA | $Cap^-$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| - | mM | | | | | | | | | | | |
| 4 | 1.00 | 4.60 | 0.32 | 0.57 | 5.23E-02 | 2.61E-02 | 5.68E-03 | 1.02E-02 | 5.49E-04 | 1.09E-04 | 9.01 | 1.16 |
| 5.5 | 1.00 | 4.60 | 0.26 | 0.46 | 4.23E-02 | 2.12E-02 | 0.15 | 0.26 | 1.41E-02 | 2.78E-03 | 8.79 | 8.07 |
| 7 | 1.00 | 4.60 | 0.11 | 0.20 | 1.81E-02 | 9.06E-03 | 1.97 | 3.52 | 0.19 | 3.77E-02 | 3.94 | 9.92 |
| 8 | 1.00 | 4.60 | 1.81E-02 | 3.23E-02 | 2.95E-03 | 1.47E-03 | 3.20 | 5.73 | 0.31 | 6.12E-02 | 0.64 | 9.99 |

*Data for Figure S6:*

**Table S29:** Caproate production using ethanol and butyrate as substrates.

| EtOH | $\Delta G_{cat}$ | AcAld | Acetyl-CoA | $Ac^-$ | Bu-CoA | Khex-CoA | Hhex-CoA | Thex-CoA | Heno-CoA | $Cap^-$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **mM** | **kJ/mol** | **mM** | | | | | | | | |
| 100.00 | -33.05 | 91.95 | 0.09 | 10.00 | 0.65 | 4.62 | 1.25 | 0.25 | 3.14 | 10.00 |
| 10.00 | -27.34 | 9.20 | 0.20 | 10.00 | 1.44 | 1.03 | 0.28 | 5.50E-02 | 7.00 | 10.00 |
| 1.00 | -21.63 | 0.92 | 0.22 | 10.00 | 1.64 | 0.12 | 3.17E-02 | 6.27E-03 | 7.98 | 10.00 |
| 0.30 | -18.65 | 0.28 | 0.23 | 10.00 | 1.66 | 3.56E-02 | 9.62E-03 | 1.90E-03 | 8.07 | 10.00 |

**Table S30:** Proton recovery and dissipation proposed in caproate production using ethanol and butyrate as substrates.

| EtOH | $\Delta G_{cat}$ | $\Delta\mu_{H+}$ in reac. 10 |
|---|---|---|
| **mM** | **kJ/mol** | **–** |
| 100.00 | -33.05 | +1 |
| 10.00 | -27.34 | +1 |
| 1.00 | -21.63 | +1 |
| 0.30 | -18.65 | +1 |

*Data for Figure S7:*

**Table S31:** Caproate production using only ethanol as a substrate.

| EtOH | $\Delta G_{cat}$ | AcAld | Acetyl-CoA | Acetyl-Pi | $Ac^-$ | AcAcetyl-CoA | HBu-CoA | Crot-CoA | Bu-CoA | Khex-CoA | Hhex-CoA | Thex-CoA | Heno-CoA | $Cap^-$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **mM** | **kJ/mol** | **mM** | | | | | | | | | | | | |
| 100.00 | -142.04 | 100.00 | 45.98 | 0.11 | 11.43 | 0.20 | 0.19 | 9.26E-02 | 8.38E-02 | 0.15 | 8.10E-02 | 1.60E-02 | 9.07 | 10.00 |
| 10.00 | -124.92 | 10.00 | 4.60 | 0.91 | 20.00 | 0.16 | 0.15 | 7.44E-02 | 6.73E-02 | 1.20E-02 | 6.51E-03 | 1.29E-03 | 8.62 | 10.00 |
| 1.00 | -107.80 | 1.00 | 0.46 | 0.16 | 13.30 | 2.91E-03 | 2.66E-03 | 1.33E-03 | 2.89 | 5.16E-02 | 2.79E-02 | 5.51E-03 | 6.86 | 10.00 |
| 0.30 | -98.84 | 0.30 | 0.14 | 0.40 | 13.24 | 2.14E-03 | 1.95E-03 | 9.75E-04 | 2.12 | 1.14E-02 | 6.15E-03 | 1.22E-03 | 7.45 | 10.00 |

**Table S32:** Proton recovery and dissipation proposed in caproate production using only ethanol as a substrate.

| EtOH | $\Delta G_{cat}$ | $\Delta G_{dis}$ in $Ac^-$ formation | $\Delta\mu_{H+}$ in reac. 6 | $\Delta\mu_{H+}$ in reac. 10 |
|---|---|---|---|---|
| **mM** | **kJ/mol** | | **—** | |
| 100.00 | -142.04 | -28 | +2 | +1 |
| 10.00 | -124.92 | -17 | +2 | +1 |
| 1.00 | -107.80 | -15 | +1 | +1 |
| 0.30 | -98.84 | -10 | +1 | +1 |

## S10. Ethanol reduction

**Table S33:** $\Delta G^0$ for acetate reduction to ethanol.

| Reaction Nº | $\Delta G^0$ (kJ/mol) |
|---|---|
| **Total** | -41.43 |
| **1** | -0.8 |
| **2** | 5.03 |
| **3** | -25.58 |
| **4** | -63.68 |

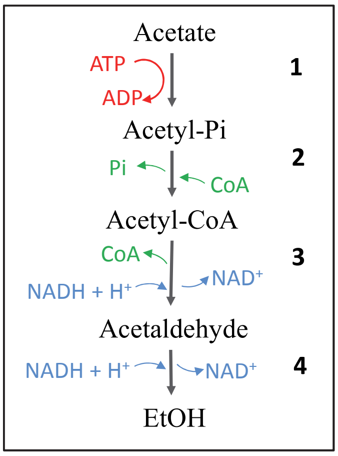

**Figure S11**[a]**:** Acetate reduction to ethanol

[a] Both reductions in this pathway can occur with NADPH instead of NADH as electron donor[8]. But for this thermodynamic analysis they are almost equivalent as they have very similar $\Delta G^0$ of formation[9].

*Data for Figure 6.a:*

**Table S34:** Acetate reduction pathway to ethanol with proton translocations at the substrate and product transport sites.

| $H_2$ | $\Delta G_{cat}$[a] | $Ac^-_{OUT}$ | $Ac^-_{IN}$ | Acetyl -Pi | Acetyl -CoA | AcAld | $EtOH_{IN}$ | $EtOH_{OUT}$ |
|---|---|---|---|---|---|---|---|---|
| **Bar** | **kJ/mol** | **mM** | | | | | | |
| 2.00 | -30.11 | 99.42 | 5.16E-06 | 6.70E-06 | 1.45E-05 | 1.33E-02 | 5.78E+05 | 0.10 |
| 3.00 | -32.12 | 99.42 | 2.30E-06 | 2.98E-06 | 6.42E-06 | 8.85E-03 | 5.78E+05 | 0.10 |
| 4.00 | -33.55 | 99.42 | 1.29E-06 | 1.67E-06 | 3.61E-06 | 6.64E-03 | 5.78E+05 | 0.10 |
| 5.00 | -34.65 | 99.42 | 8.26E-07 | 1.07E-06 | 2.31E-06 | 5.31E-03 | 5.78E+05 | 0.10 |

[a] Global Gibbs energy considering the reaction ($Ac^- + 2H_2 + H^+ \rightarrow EtOH + H_2O$). 50 kJ/mol extra are invested in the catabolic process with the hydrolysis of one ATP.

**Table S35:** Proton motive force recovered proposed for anabolism and maintenance in the acetate reduction pathway to ethanol.

| $H_2$ | $\Delta G_{cat}$ | $\Delta\mu_{H+}$ in $Ac^-$ Transp. | $\Delta\mu_{H+}$ in EtOH Transp. |
|---|---|---|---|
| **Bar** | **kJ/mol** | – | |
| 2.00 | -30.11 | +2 | +2 |
| 3.00 | -32.12 | +2 | +2 |
| 4.00 | -33.55 | +2 | +2 |
| 5.00 | -34.65 | +2 | +2 |

*Data for Figure 6.b:*

**Table S36:** Acetate reduction pathway to ethanol with recovery of ATP via SLP.

| $H_2$ | $\Delta G_{cat}$ | $Ac^{-}_{OUT}$ | $Ac^{-}_{IN}$ | Acetyl -Pi | Acetyl -CoA | AcAld | $EtOH_{IN}$ | $EtOH_{OUT}$ |
|---|---|---|---|---|---|---|---|---|
| **Bar** | **kJ/mol** | **mM** | | | | | | |
| 2.00 | -30.11 | 99.42 | 5.15E-04 | 6.67E-04 | 2.50E-12 | 2.30E-09 | 0.10 | 0.10 |
| 3.00 | -32.12 | 99.42 | 2.29E-04 | 2.97E-04 | 1.11E-12 | 1.53E-09 | 0.10 | 0.10 |
| 4.00 | -33.55 | 99.42 | 1.29E-04 | 1.67E-04 | 6.25E-13 | 1.15E-09 | 0.10 | 0.10 |
| 5.00 | -34.65 | 99.42 | 8.23E-05 | 1.07E-04 | 4.00E-13 | 9.20E-10 | 0.10 | 0.10 |

**Table S37:** Proton motive force recovered proposed for anabolism and maintenance in the acetate reduction pathway to ethanol.

| $H_2$ | $\Delta G_{cat}$ | $\Delta\mu_{H+}$ in $Ac^{-}$ Transp. |
|---|---|---|
| **Bar** | **kJ/mol** | – |
| 2.00 | -30.11 | +1 |
| 3.00 | -32.12 | +1 |
| 4.00 | -33.55 | +1 |
| 5.00 | -34.65 | +1 |

*Data for Figure 6.c:*

**Table S38:** Acetate reduction pathway to ethanol with proton translocations at the substrate transport site and at two reduction sites.

| $H_2$ | $\Delta G_{cat}$ | $Ac^{-}_{OUT}$ | $Ac^{-}_{IN}$ | Acetyl -Pi | Acetyl -CoA | AcAld | $EtOH_{IN}$ | $EtOH_{OUT}$ |
|---|---|---|---|---|---|---|---|---|
| **Bar** | **kJ/mol** | **mM** | | | | | | |
| 2.00 | -30.11 | 99.42 | 1.24E-02 | 1.61E-02 | 3.46E-02 | 1.33E-02 | 0.10 | 0.10 |
| 3.00 | -32.12 | 99.42 | 5.52E-03 | 7.15E-03 | 1.54E-02 | 8.85E-03 | 0.10 | 0.10 |
| 4.00 | -33.55 | 99.42 | 3.10E-03 | 4.02E-03 | 8.68E-03 | 6.64E-03 | 0.10 | 0.10 |
| 5.00 | -34.65 | 99.42 | 1.99E-03 | 2.58E-03 | 5.56E-03 | 5.31E-03 | 0.10 | 0.10 |

**Table S39:** Proton motive force recovered proposed for anabolism and maintenance in the acetate reduction pathway to ethanol.

| $H_2$ | $\Delta G_{cat}$ | $\Delta\mu_{H+}$ in $Ac^{-}$ Transp. | $\Delta\mu_{H+}$ in reac. 3 | $\Delta\mu_{H+}$ in reac. 4 |
|---|---|---|---|---|
| **Bar** | **kJ/mol** | – | | |
| 2.00 | -30.11 | +1 | +1 | +2 |
| 3.00 | -32.12 | +1 | +1 | +2 |
| 4.00 | -33.55 | +1 | +1 | +2 |
| 5.00 | -34.65 | +1 | +1 | +2 |

*Data for Figure 6.d:*

**Table S40:** Acetate reduction pathway to ethanol with proton translocations at the two reduction sites only.

| $H_2$ | $\Delta G_{cat}$ | $Ac^-_{OUT}$ | $Ac^-_{IN}$ | Acetyl -Pi | Acetyl -CoA | AcAld | $EtOH_{IN}$ | $EtOH_{OUT}$ |
|---|---|---|---|---|---|---|---|---|
| **Bar** | **kJ/mol** | **mM** | | | | | | |
| 2.00 | -30.11 | 99.42 | 29.84 | 7.95 | 8.93 | 31.93 | 0.10 | 0.10 |
| 3.00 | -32.12 | 99.42 | 13.26 | 6.32 | 7.88 | 21.28 | 0.10 | 0.10 |
| 4.00 | -33.55 | 99.42 | 7.46 | 4.92 | 6.76 | 15.96 | 0.10 | 0.10 |
| 5.00 | -34.65 | 99.42 | 4.77 | 3.82 | 5.72 | 12.77 | 0.10 | 0.10 |

**Table S41:** Proton motive force recovered proposed for anabolism and maintenance in the acetate reduction pathway to ethanol.

| $H_2$ | $\Delta G_{cat}$ | $\Delta G_{dis}$ in $Ac^-$ Transp. | $\Delta\mu_{H+}$ in reac. 3 | $\Delta\mu_{H+}$ in reac. 4 |
|---|---|---|---|---|
| **Bar** | **kJ/mol** | | **—** | |
| 2.00 | -30.11 | -2.98 | +1 | +3 |
| 3.00 | -32.12 | -4.99 | +1 | +3 |
| 4.00 | -33.55 | -6.42 | +1 | +3 |
| 5.00 | -34.65 | -7.52 | +1 | +3 |

*Data for Figure S5.a:*

**Table S42:** Acetate reduction pathway to ethanol with different pH and a constant potential gradient across the membrane of -200 mV.

| pH | $H_2$ | $\Delta G_{cat}$ | $Ac^-_{OUT}$ | $Ac^-_{IN}$ | Acetyl -Pi | Acetyl -CoA | AcAld | $EtOH_{IN}$ | $EtOH_{OUT}$ |
|---|---|---|---|---|---|---|---|---|---|
| - | **Bar** | **kJ/mol** | **mM** | | | | | | |
| 5 | 2.00 | -40.41 | 63.27 | 2.98E-07 | 3.87E-07 | 8.35E-07 | 3.19E-05 | 0.10 | 0.10 |
| 6 | 2.00 | -35.70 | 94.51 | 2.98E-03 | 3.87E-03 | 8.34E-03 | 3.19E-02 | 0.10 | 0.10 |
| 7 | 2.00 | -30.11 | 99.42 | 29.84 | 7.95 | 8.93 | 31.93 | 0.10 | 0.10 |
| 8 | 2.00 | -24.41 | 99.94 | 2.98E+05 | 10.00 | 10.00 | 3.19E+04 | 0.10 | 0.10 |

*Data for Figure S5.b:*

**Table S43:** Acetate reduction pathway to ethanol with different pH optimizing the membrane potential.

| pH | $\Delta\Psi$ | $H_2$ | $\Delta G_{cat}$ | $Ac^-_{OUT}$ | $Ac^-_{IN}$ | Acetyl -Pi | Acetyl -CoA | AcAld | $EtOH_{IN}$ | $EtOH_{OUT}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| - | **mV** | **Bar** | **kJ/mol** | **mM** | | | | | | |
| 5 | -80 | 2.00 | -40.41 | 63.27 | 22.95 | 7.48 | 8.65 | 1.16E-02 | 0.10 | 0.10 |
| 6 | -140 | 2.00 | -35.70 | 94.51 | 11.63 | 6.01 | 7.65 | 8.29E-03 | 0.10 | 0.10 |
| 7 | -200 | 2.00 | -30.11 | 99.42 | 7.46 | 4.92 | 6.76 | 6.64E-03 | 0.10 | 0.10 |
| 8 | -260 | 2.00 | -24.41 | 99.94 | 5.44 | 4.14 | 6.04 | 5.67E-03 | 0.10 | 0.10 |

*Data for Figure S8:*

**Table S44:** Acetate reduction pathway to ethanol with proton translocations at the two reduction sites only.

| $H_2$ | $\Delta G_{cat}$ | $Ac^-_{OUT}$ | $Ac^-_{IN}$ | Acetyl -Pi | Acetyl -CoA | AcAld | $EtOH_{IN}$ | $EtOH_{OUT}$ |
|---|---|---|---|---|---|---|---|---|
| **Bar** | **kJ/mol** | **mM** | | | | | | |
| 2.00 | -30.11 | 99.42 | 29.84 | 7.95 | 8.93 | 1.33E-02 | 0.10 | 0.10 |
| 3.00 | -32.12 | 99.42 | 13.26 | 6.32 | 7.88 | 8.85E-03 | 0.10 | 0.10 |
| 4.00 | -33.55 | 99.42 | 7.46 | 4.92 | 6.76 | 6.64E-03 | 0.10 | 0.10 |
| 5.00 | -34.65 | 99.42 | 4.77 | 3.82 | 5.72 | 5.31E-03 | 0.10 | 0.10 |

**Table S45:** Proton motive force recovered proposed for anabolism and maintenance in the acetate reduction pathway to ethanol.

| $H_2$ | $\Delta G_{cat}$ | $\Delta G_{dis}$ in $Ac^-$ Transp. | $\Delta\mu_{H+}$ in reac. 3 | $\Delta\mu_{H+}$ in reac. 4 |
|---|---|---|---|---|
| **Bar** | **kJ/mol** | | **–** | |
| 2.00 | -30.11 | -2.98 | +2 | +2 |
| 3.00 | -32.12 | -4.99 | +2 | +2 |
| 4.00 | -33.55 | -6.42 | +2 | +2 |
| 5.00 | -34.65 | -7.52 | +2 | +2 |

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