Fundamentals of enzyme kinetics and thermodynamic analysis for microbial communities (2nd Part)

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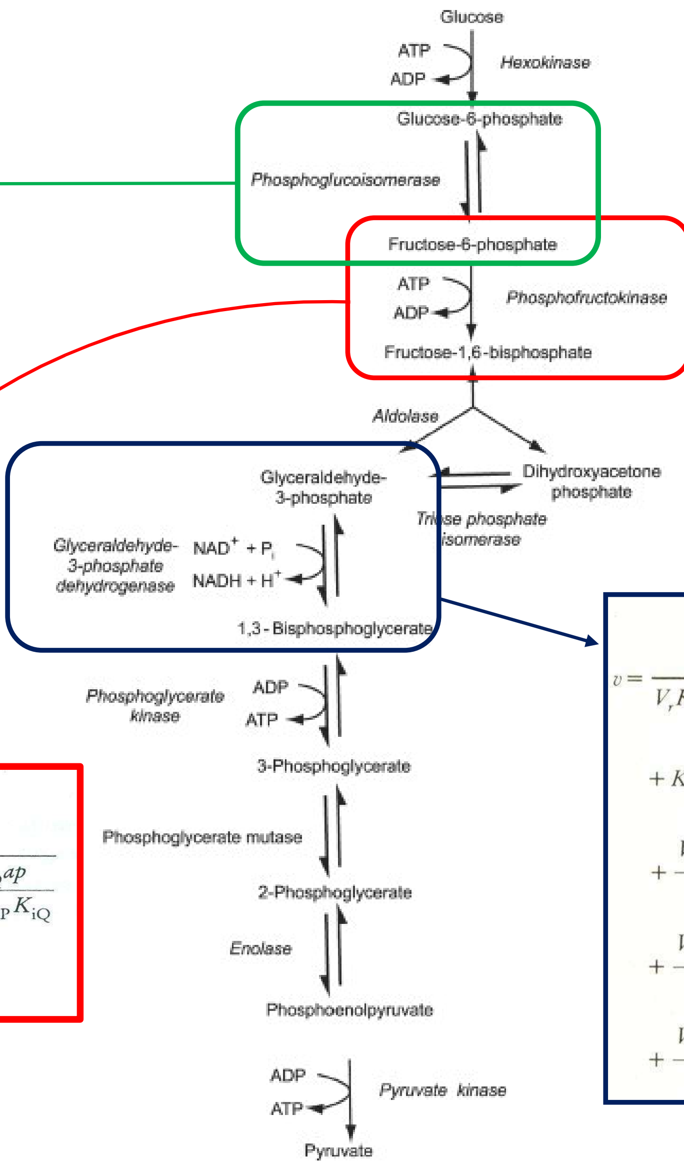
One of my favorite phrases:

ROME WAS NOT CONSTRUCTED IN ONE DAY

Thermodynamic analysis of the metabolism cannot be understood in 2.5 hours

9:00 – 11:30 Thermodynamics and computational practice

$$v = \frac{\frac{k_{cat}^{f}}{K_s} * E * s - \frac{k_{cat}^{r}}{K_p} * E * p}{1 + \frac{s}{K_s} + \frac{p}{K_p}}$$



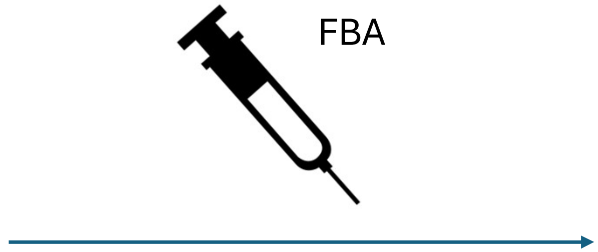
$$v = \frac{\frac{V_{+}ab}{K_{iA}K_{mB}} - \frac{V_{-}pq}{K_{mP}K_{iQ}}}{1 + \frac{a}{K_{iA}} + \frac{K_{mA}b}{K_{iA}K_{mB}} + \frac{K_{mQ}p}{K_{mP}K_{iQ}} + \frac{q}{K_{iQ}} + \frac{ab}{K_{iA}K_{mB}} + \frac{K_{mQ}ap}{K_{iA}K_{mP}K_{iQ}} + \frac{K_{mA}bq}{K_{iA}K_{mB}K_{iQ}} + \frac{pq}{K_{mP}K_{iQ}} + \frac{abp}{K_{iA}K_{mB}K_{iP}} + \frac{bpq}{K_{iB}K_{mP}K_{iQ}}}$$

$$v = \frac{V_f V_r\left([A][B][C] - \frac{[P][Q]}{K_{eq}}\right)}{\begin{aligned} & V_r K_{ia}K_{ib}K_{m_C} + V_r K_{ib}K_{m_C}[A] + V_r K_{ia}K_{m_B}[C] + V_r K_{m_C}[A][B] \\ & + K_{m_B}[A][C] + V_r K_{m_A}[B][C] + V_r[A][B][C] + \frac{V_f K_{m_Q}[P]}{K_{eq}} \\ & + \frac{V_f K_{m_P}[Q]}{K_{eq}} + \frac{V_f[P][Q]}{K_{eq}} + \frac{V_f K_{m_Q}[A][P]}{K_{ia}K_{eq}} + \frac{V_f K_{m_Q}[A][B][P]}{K_{ia}K_{ib}K_{eq}} \\ & + \frac{V_f K_{m_Q}[A][B][C][P]}{K_{ia}K_{ib}K_{ic}K_{eq}} + \frac{V_r K_{ia}K_{m_B}[C][Q]}{K_{iq}} + \frac{V_r K_{m_A}[B][C][Q]}{K_{iq}} \\ & + \frac{V_r K_{ia}K_{m_B}[C][P][Q]}{K_{ip}K_{iq}} + \frac{V_r K_{m_A}K_{ic}[B][P][Q]}{K_{ip}K_{iq}} + \frac{V_r K_{m_A}[B][C][P][Q]}{K_{ip}K_{iq}} \end{aligned}}$$

We already have some elements that help us to understand/simulate parts of this (very complex) problem:

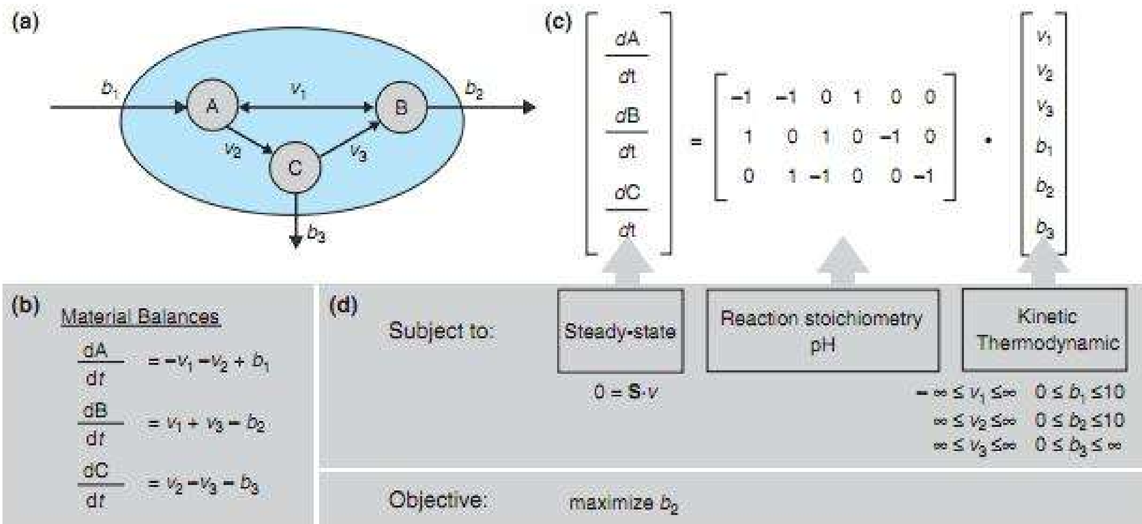
- We can represent irreversible Uni-Uni reactions
- We can represent irreversible transporters
- With Timmy: you learnt how to calculate metabolic fluxes under steady-state conditions

Flux Balances Analysis (FBA) simplifies enormously the metabolic network analysis



However,....

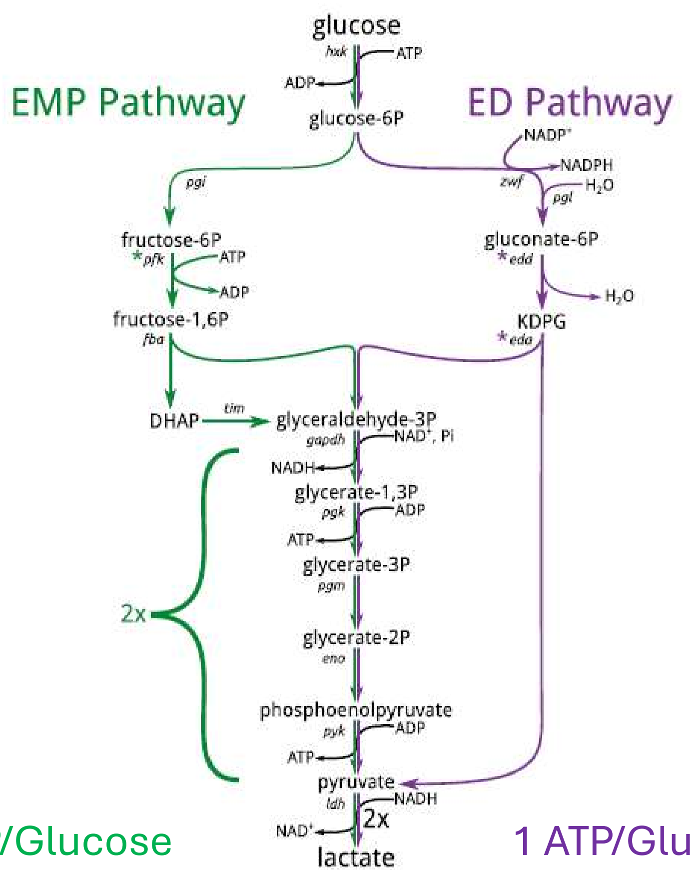
FBA is “blind” to metabolite concentrations



- Enzyme inhibition, activation, inactivation depend on metabolite concentrations
- The direction of metabolic fluxes depends on metabolite concentrations

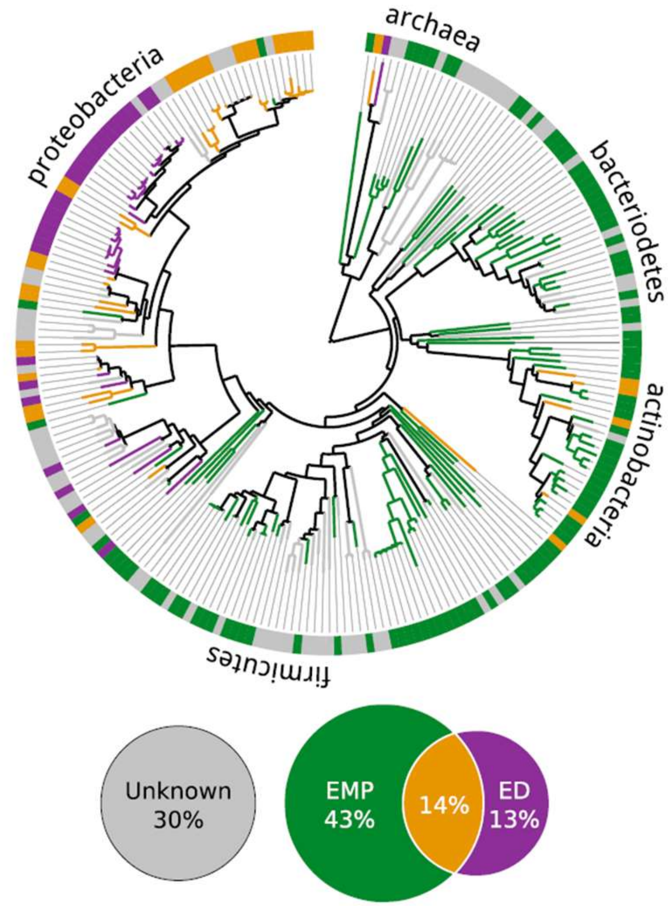
Glycolytic strategy as a tradeoff between energy yield and protein cost

Avi Flamholz[a,1], Elad Noor[a,1], Arren Bar-Even[a], Wolfram Liebermeister[a,b], and Ron Milo[a,2]



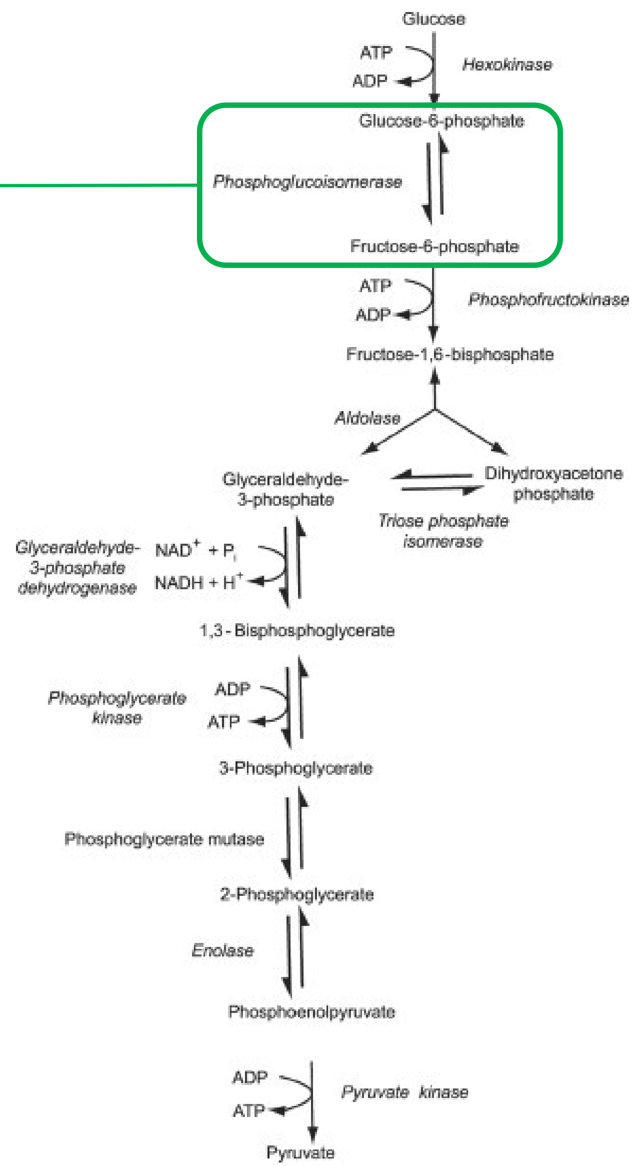
2 ATP/Glucose

1 ATP/Glucose



FBA alone cannot explain why so many microorganisms use the Entner-Doudoroff pathway

$$v = \frac{\frac{k_{cat}^{f}}{K_s} * E * s - \frac{k_{cat}^{r}}{K_p} * E * p}{1 + \frac{s}{K_s} + \frac{p}{K_p}}$$



$$E + S \underset{k_{1r}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{2r}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Assuming **steady-state** for the enzyme-substrate complex:

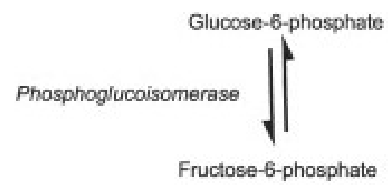
$$\frac{dES}{dt} = 0 \qquad k_{cat}^{f} = k_2 \qquad K_M^S = \frac{k_{1r} + k_2}{k_1} \qquad k_{cat}^{r} = k_{1r} \qquad K_M^P = \frac{k_{1r} + k_2}{k_{2r}}$$

Haldane's equation

$$\frac{dP}{dt} = -\frac{dS}{dt} = \frac{\frac{k_{cat}^{f}}{K_M^S} * E * S(t) - \frac{k_{cat}^{r}}{K_M^P} * E * P(t)}{1 + \frac{S(t)}{K_M^S} + \frac{P(t)}{K_M^P}}$$

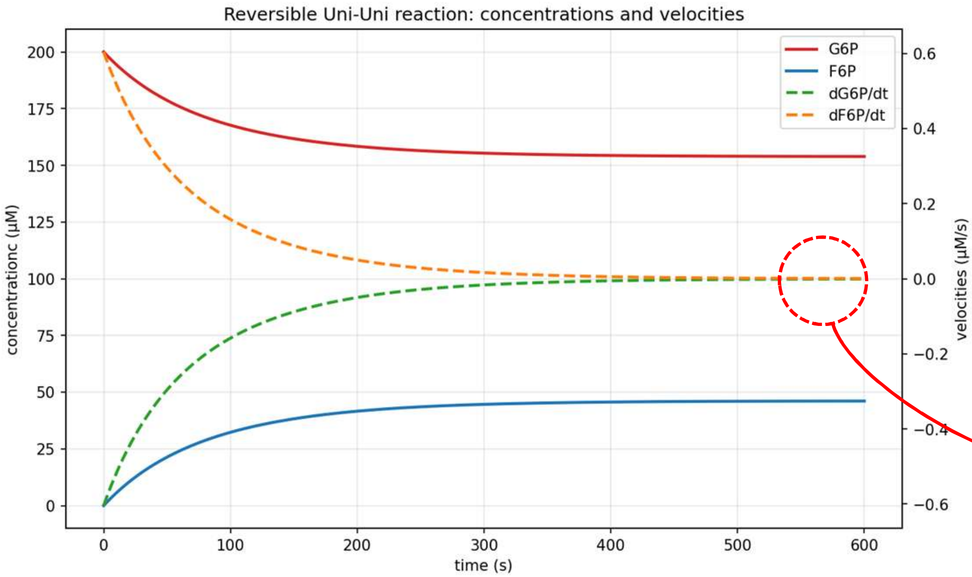
Applying the Haldane's equation to the reaction catalyzed by the phosphoglucose isomerase

$$\frac{dP}{dt} = -\frac{dS}{dt} = \frac{\frac{k_{cat}^{f}}{K_M^S} * E * S(t) - \frac{k_{cat}^{r}}{K_M^P} * E * P(t)}{1 + \frac{S(t)}{K_M^S} + \frac{P(t)}{K_M^P}}$$



$$\frac{dF6P}{dt} = -\frac{dG6P}{dt} = \frac{\frac{k_{cat}^{f,PGI}}{K_M^{G6P,PGI}} * \mathrm{PGI} * G6P(t) - \frac{k_{cat}^{r,PGI}}{K_M^{F6P,PGI}} * \mathrm{PGI} * F6P(t)}{1 + \frac{G6P(t)}{K_M^{G6P,PGI}} + \frac{F6P(t)}{K_M^{F6P,PGI}}}$$

Let's imagine that the reaction goes until approaching equilibrium:



$$\frac{dF6P}{dt} = -\frac{dG6P}{dt} = \frac{\frac{k_{cat}^{f,PGI}}{K_M^{G6P,PGI}} * \mathrm{PGI} * G6P(t) - \frac{k_{cat}^{r,PGI}}{K_M^{F6P,PGI}} * \mathrm{PGI} * F6P(t)}{1 + \frac{G6P(t)}{K_M^{G6P,PGI}} + \frac{F6P(t)}{K_M^{F6P,PGI}}}$$

$$\frac{dF6P}{dt} = -\frac{dG6P}{dt} = \frac{\frac{k_{cat}^{f,PGI}}{K_M^{G6P,PGI}} * \mathrm{PGI} * G6P^{eq} - \frac{k_{cat}^{r,PGI}}{K_M^{F6P,PGI}} * \mathrm{PGI} * F6P^{eq}}{1 + \frac{G6P^{eq}}{K_M^{G6P,PGI}} + \frac{F6P^{eq}}{K_M^{F6P,PGI}}} = 0$$

$$\frac{dF6P}{dt} = -\frac{dG6P}{dt} = \frac{\frac{k_{cat}^{f,PGI}}{K_M^{G6P,PGI}} * \mathrm{PGI} * G6P^{eq} - \frac{k_{cat}^{r,PGI}}{K_M^{F6P,PGI}} * \mathrm{PGI} * F6P^{eq}}{1 + \frac{G6P^{eq}}{K_M^{G6P,PGI}} + \frac{F6P^{eq}}{K_M^{F6P,PGI}}} = 0$$

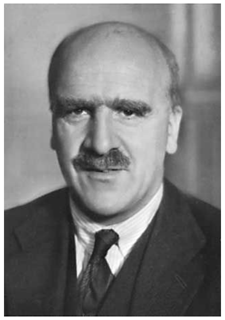
$$\frac{k_{cat}^{f,PGI}}{K_M^{G6P,PGI}} * \cancel{PGI} * G6P^{eq} = \frac{k_{cat}^{r,PGI}}{K_M^{F6P,PGI}} * \cancel{PGI} * F6P^{eq}$$

$$\frac{F6P^{eq}}{G6P^{eq}} = K_{eq}^{PGI} = \frac{k_{cat}^{f,PGI} * K_M^{F6P,PGI}}{k_{cat}^{r,PGI} * K_M^{G6P,PGI}}$$

$$\frac{F6P^{eq}}{G6P^{eq}} = K_{eq}^{PGI} = \frac{k_{cat}^{f,PGI} * K_M^{F6P,PGI}}{k_{cat}^{r,PGI} * K_M^{G6P,PGI}}$$

Haldane's relationship

$$\frac{P^{eq}}{S^{eq}} = K_{eq} = \frac{k_{cat}^{f} * K_M^{P}}{k_{cat}^{r} * K_M^{S}}$$



https://en.wikipedia.org/wiki/J._B._S._Haldane

Main considerations regarding the Haldane relationship:

$$K_{eq} = \frac{k_{cat}^{f} * K_{M}^{P}}{k_{cat}^{r} * K_{M}^{S}}$$

The kinetic parameters of the reaction in the forward direction **are not independent** of the kinetic parameters of the reaction in the backward direction.

If either by evolution or protein engineering, the $k_{cat}{}^{f}$ of a reaction is modified, this change necessarily implies a change in at least one of the other kinetic parameters

Main considerations regarding the Haldane relationship:

More complex enzyme-catalyzed reactions also have their corresponding Haldane relationships.

$$v = \frac{\frac{k_{cat}^f}{K_s} * E * s - \frac{k_{cat}^r}{K_p} * E * p}{1 + \frac{s}{K_s} + \frac{p}{K_p}}$$

$$K_{eq} = \frac{k_{cat}^f * K_M^P}{k_{cat}^r * K_M^S}$$

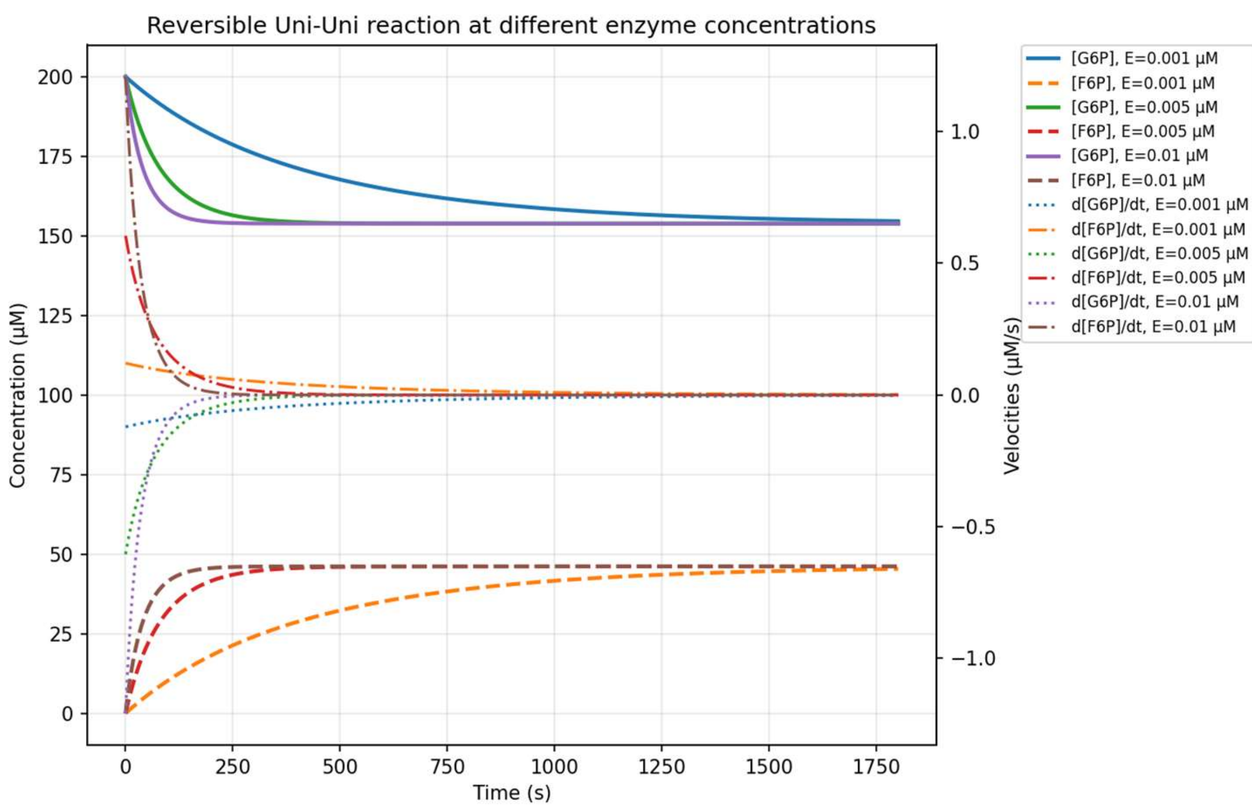
$$v = \frac{\frac{V_+ ab}{K_{iA} K_{mB}} - \frac{V_- pq}{K_{mP} K_{iQ}}}{1 + \frac{a}{K_{iA}} + \frac{K_{mA} b}{K_{iA} K_{mB}} + \frac{K_{mQ} p}{K_{mP} K_{iQ}} + \frac{q}{K_{iQ}} + \frac{ab}{K_{iA} K_{mB}} + \frac{K_{mQ} ap}{K_{iA} K_{mP} K_{iQ}} + \frac{K_{mA} bq}{K_{iA} K_{mB} K_{iQ}} + \frac{pq}{K_{mP} K_{iQ}} + \frac{abp}{K_{iA} K_{mB} K_{iP}} + \frac{bpq}{K_{iB} K_{mP} K_{iQ}}}$$

$$K_{eq} = \frac{k_{cat}^f * K_M^P * K_M^Q}{k_{cat}^r * K_M^A * K_M^B}$$

$$v = \frac{V_f V_r \left([A][B][C] - \frac{[P][Q]}{K_{eq}} \right)}{\begin{aligned} & V_r K_{ia} K_{ib} K_{m_C} + V_r K_{ib} K_{m_C}[A] + V_r K_{ia} K_{m_B}[C] + V_r K_{m_C}[A][B] \\ & + K_{m_B}[A][C] + V_r K_{m_A}[B][C] + V_r [A][B][C] + \frac{V_f K_{m_Q}[P]}{K_{eq}} \\ & + \frac{V_f K_{m_P}[Q]}{K_{eq}} + \frac{V_f [P][Q]}{K_{eq}} + \frac{V_f K_{m_Q}[A][P]}{K_{ia} K_{eq}} + \frac{V_f K_{m_Q}[A][B][P]}{K_{ia} K_{ib} K_{eq}} \\ & + \frac{V_f K_{m_Q}[A][B][C][P]}{K_{ia} K_{ib} K_{ic} K_{eq}} + \frac{V_r K_{ia} K_{m_B}[C][Q]}{K_{iq}} + \frac{V_r K_{m_A}[B][C][Q]}{K_{iq}} \\ & + \frac{V_r K_{ia} K_{m_B}[C][P][Q]}{K_{ip} K_{iq}} + \frac{V_r K_{m_A} K_{ic}[B][P][Q]}{K_{ip} K_{iq}} + \frac{V_r K_{m_A}[B][C][P][Q]}{K_{ip} K_{iq}} \end{aligned}}$$

$$K_{eq} = \frac{k_{cat}^f * K_M^P * K_M^Q}{k_{cat}^r * K_M^A * K_M^B * K_M^C}$$

The enzyme concentration determines how fast we approach the equilibrium, but enzyme concentration cannot change the concentrations of substrates and products at the equilibrium



$$\Delta_r G = \Delta_r G^o + RT * \ln Q$$

$\Delta_r G^o$: released free energy when the reaction is at given standard condition, typically expressed in kJ/mol

R : Gas constant 0.008314 kJ/mol/K

T: temperature, in Kelvin.

Q: mass-action ratio

$$Q = \frac{\prod_{P_1}^{P_n} [P]^{stoichiometric\ coefficient}}{\prod_{S_1}^{S_m} [S]^{stoichiometric\ coefficient}}$$

$$\text{F6}P + ATP \leftrightarrow FBP + ADP \qquad Q = \frac{[FBP]*[ADP]}{[F6P]*[ATP]}$$

$$2\ AcCoA \leftrightarrow AcAcCoA + CoA \qquad Q = \frac{[AcAcCoA]*[CoA]}{AcCoA^2}$$

$\Delta_r G$ indicates us if the reaction is thermodynamically feasible or not:

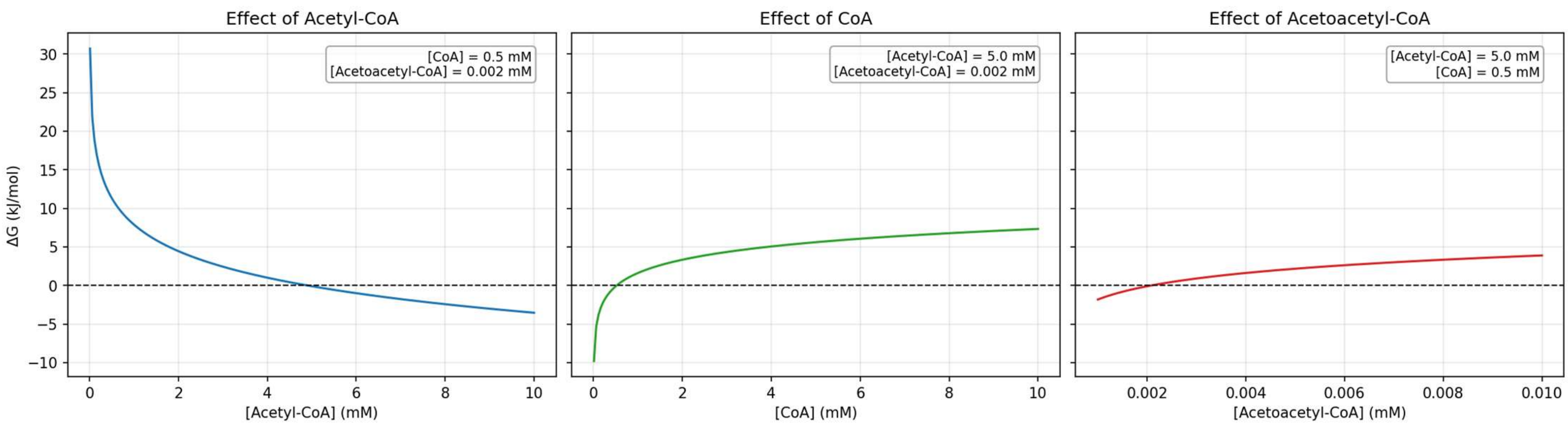
$\Delta_r G$<0: thermodynamically feasible

$\Delta_r G$≥0: thermodynamically infeasible

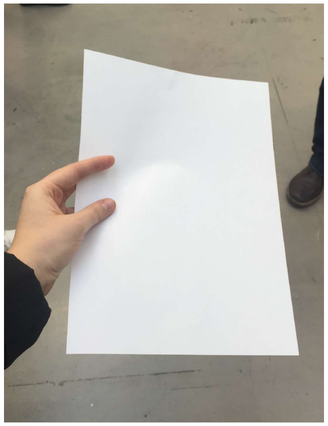
$$2\ AcCoA \leftrightarrow AcAcCoA + CoA$$

$\Delta_r G^o$ = 25 kJ/mol, T = 298.15 K

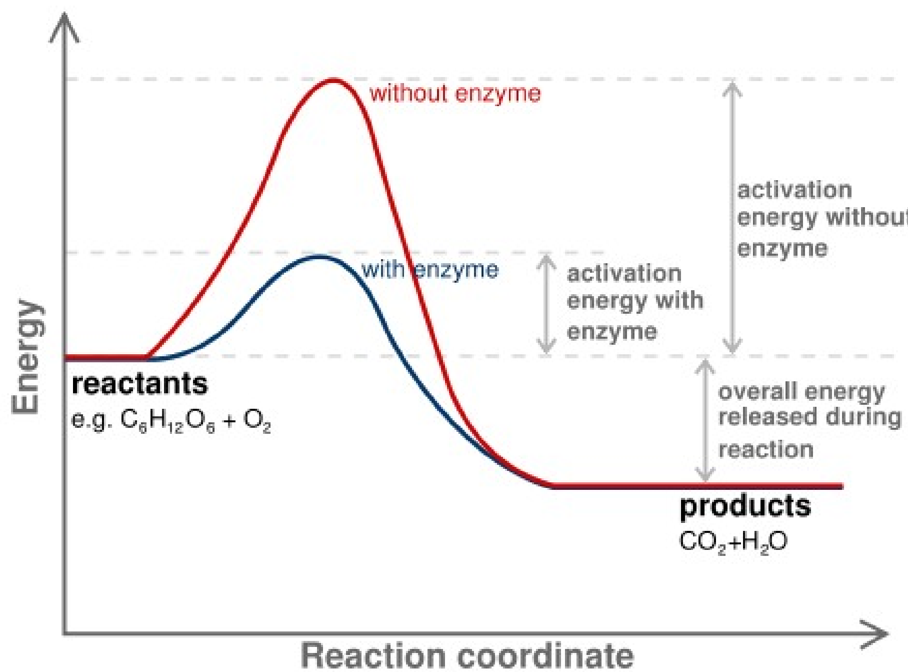
$$\Delta_r G^{AAR} = 25\ \text{kJ/mol} + 0.0083\ \text{kJ/mol/K} * 298.15\ \text{K} * \ln \frac{[AcAcCoA]*[CoA]}{AcCoA^2}$$



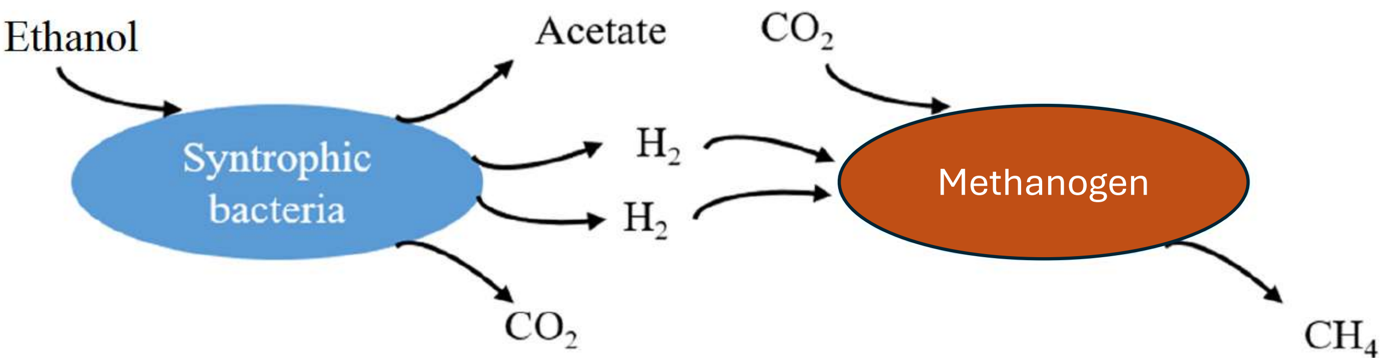
But ***feasible*** does not mean that ***actually happens***!!



Enzymes decrease the activation energy



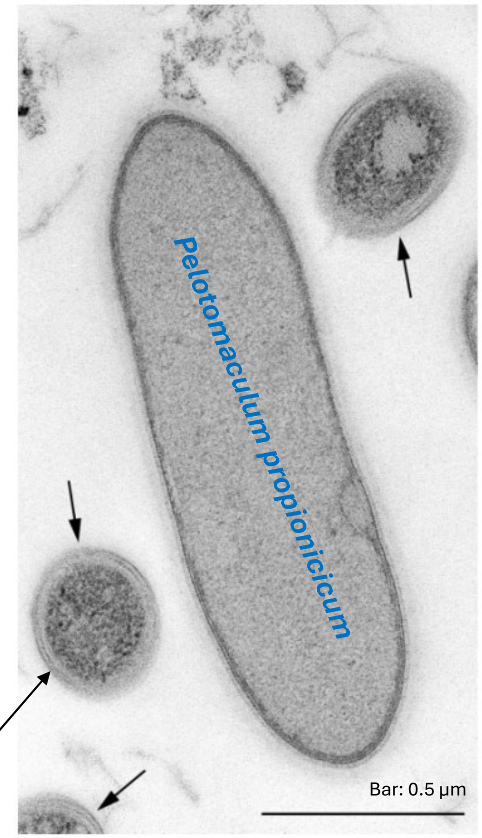
Syntrophic relationship between an acetogen and a methanogen



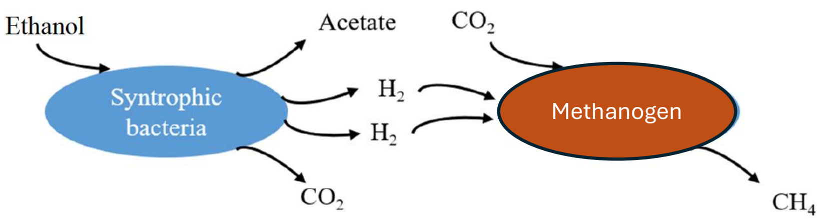
Acetogen: Ethanol + $H_2O \rightarrow$ Acetate + 2 H_2 ($\Delta G'^{\circ}$ = +40 kJ/mol)

Methanogen: 4 H_2 + $CO_2 \rightarrow$ Methane + 2 H_2O ($\Delta G'^{\circ}$ = -195 kJ/mol)

Methanospirillum hungatei

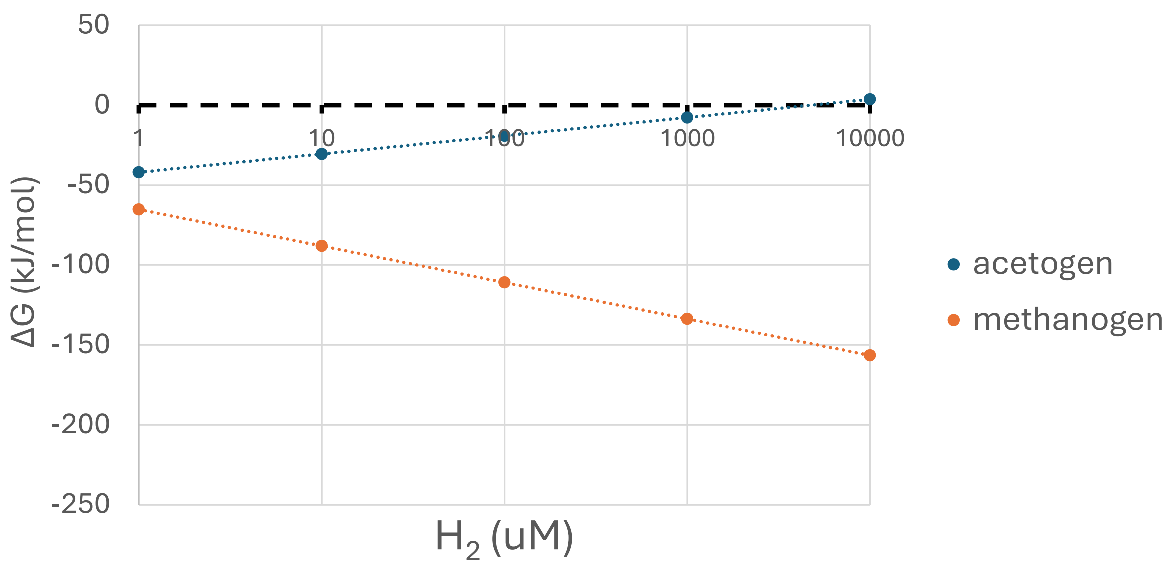


Source: DOI 10.1099/ijs.0.64925-0



$$\Delta rG^{\,acetogen} = \Delta rG^{\circ\,acetogen} + RT \ln \frac{[H_2]^2 * [Acetate]}{[Ethanol]}$$

$$\Delta rG^{methanogen} = \Delta rG^{\circ\,methanogen} + RT \ln \frac{[Methane]}{[H_2]^4 * [CO_2]}$$

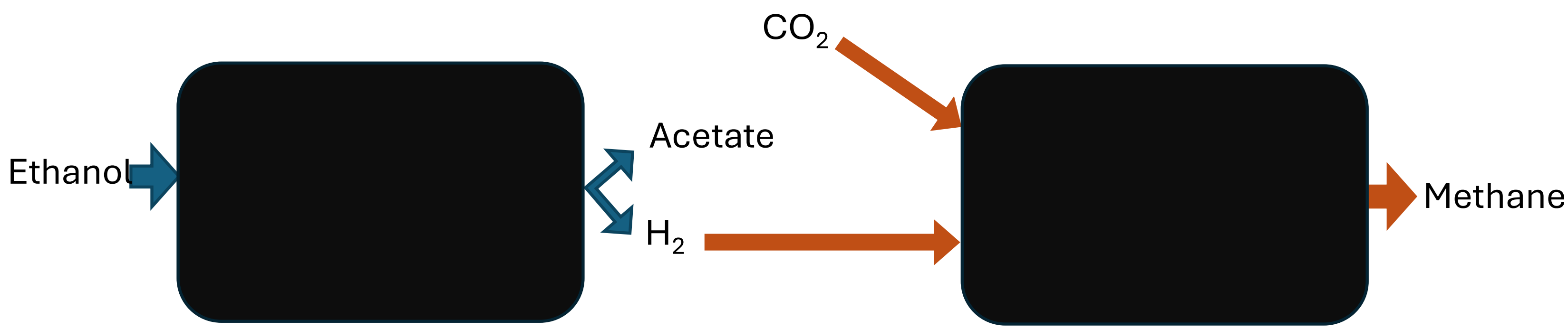


H_2 concentration must be below a certain threshold to allow the survival of the acetogen

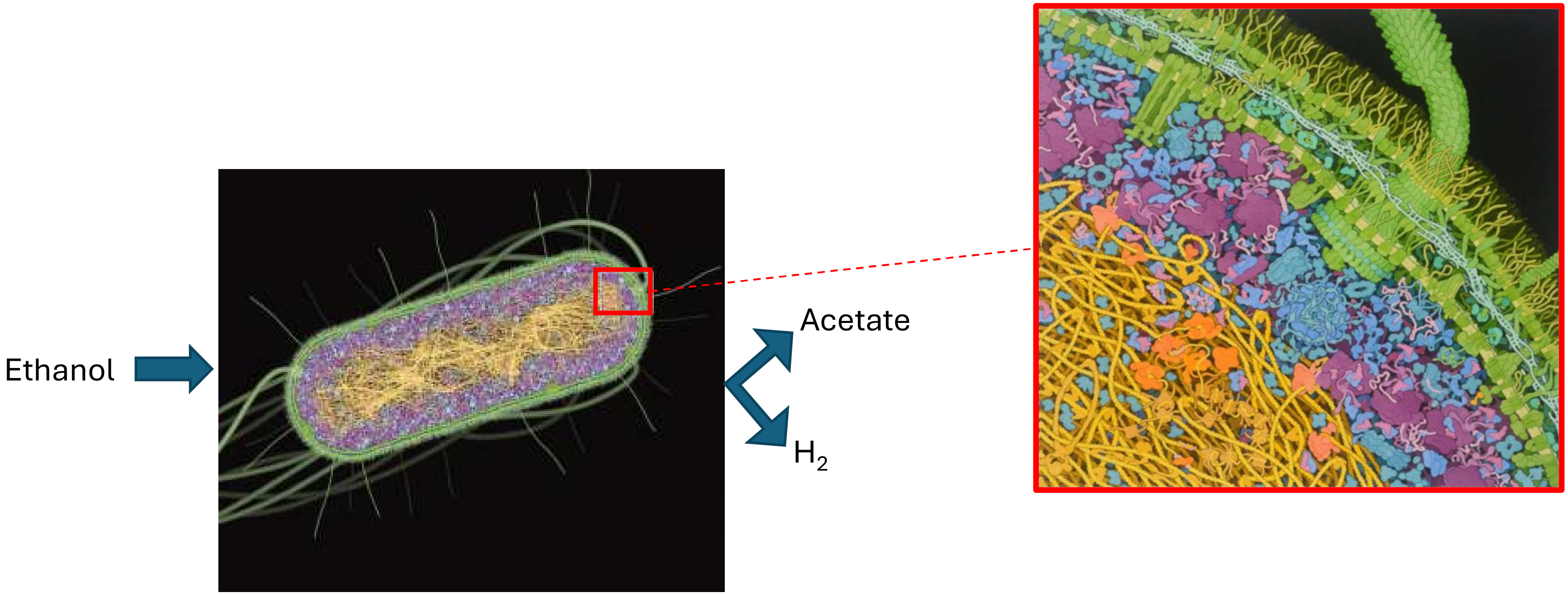
We can understand the feasibility of this syntrophic relationship using the black box perspective

$$\Delta rG^{\,acetogen} = \Delta rG^{\circ\,acetogen} + RT \ln \frac{[H_2]^2 * [Acetate]}{[Ethanol]}$$

$$\Delta rG^{methanogen} = \Delta rG^{\circ\,methanogen} + RT \ln \frac{[Methane]}{[H_2]^4 * [CO_2]}$$



Looking inside the black box: the "reaction" is ***much*** more complex



$$\text{Ethanol} + H_2O \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \text{Acetate} + 2\,H_2$$

Source: doi: 10.2210/rcsb_pdb/goodsell-gallery-028

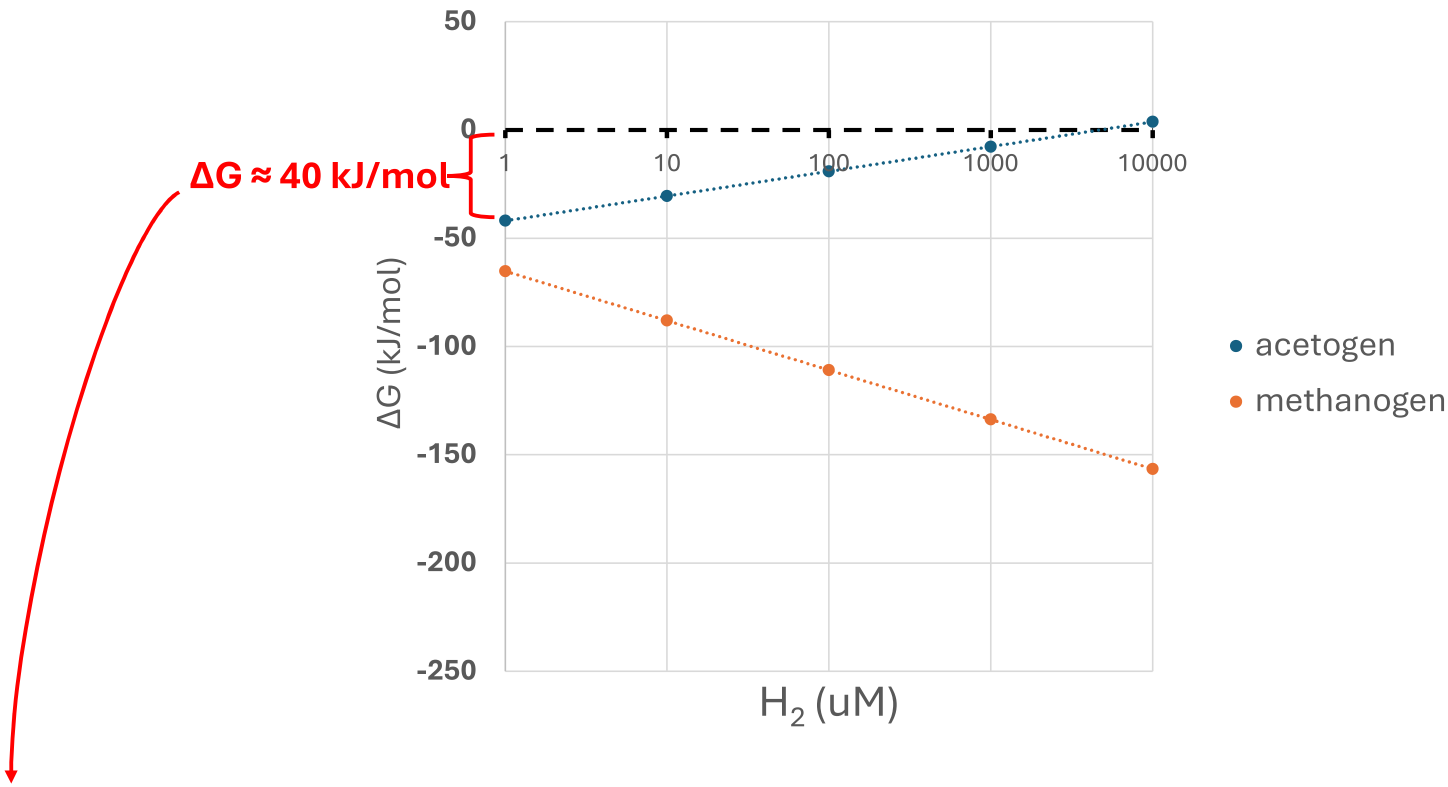
Carbon + electron source → → → → → → → → → → → Products

Metabolites **Energy**

Stoichiometric analysis

focus today

Ethanol + H_2O → → → → → → → → → → → Acetate + 2 H_2



From the *40 kJ per mol of consumed ethanol*, the acetogenic bacteria should be able to extract the energy to drive its cellular processes

How much is **40 kJ/mol**?

"adjusted" concentrations

All concentrations at 1 mM

All concentrations at 1 M

https://equilibrator.weizmann.ac.il/

The larger amount of ATP produced aerobically versus anaerobically has an obvious thermodynamic explanation

Glucose(aq) $\rightleftharpoons$ 2 Ethanol(aq) + 2 CO2(aq)

Reaction Gibbs Energy		
Estimated $\Delta_r G'^m$	-266.1 ± 12.8 [kJ/mol]	
Estimated $\Delta_r G'^\circ$	-214.8 ± 12.8 [kJ/mol]	$K'_{eq} = 4.5 \times 10^{37}$

Glucose(aq) + 6 O2(aq) $\rightleftharpoons$ 6 H2O(l) + 6 CO2(aq)

Reaction Gibbs Energy		
Estimated $\Delta_r G'^m$	-2910.6 ± 49.5 [kJ/mol]	
Estimated $\Delta_r G'^\circ$	-2927.8 ± 49.5 [kJ/mol]	$K'_{eq} = 1.6 \times 10^{513}$

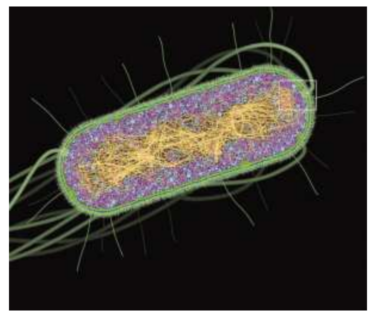
Five minutes break

Living beings have mechanisms to conserve part of the available $\Delta_r G$

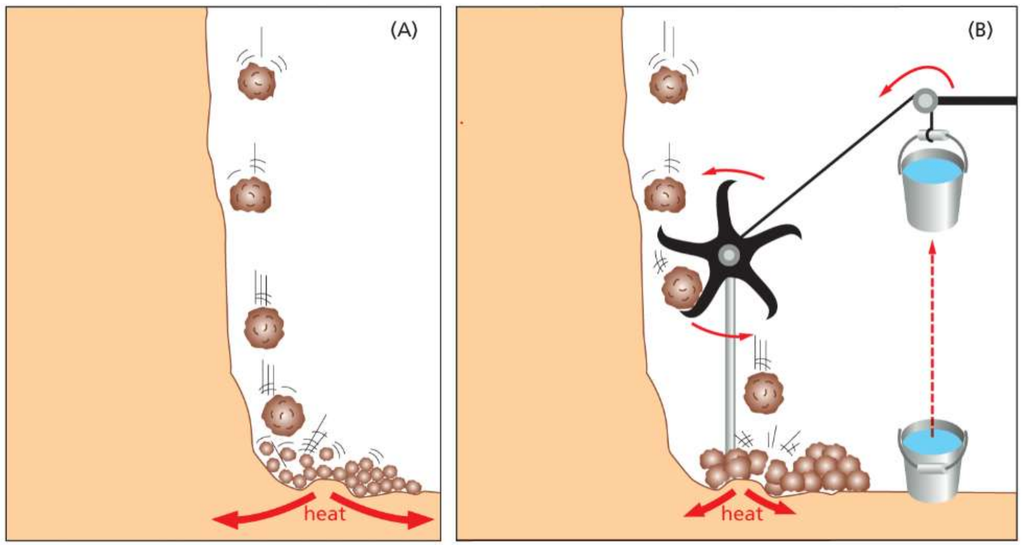
Cellulose + O_2 → CO_2 + H_2O

Glucose + O_2 - - - → CO_2 + H_2O

ATP



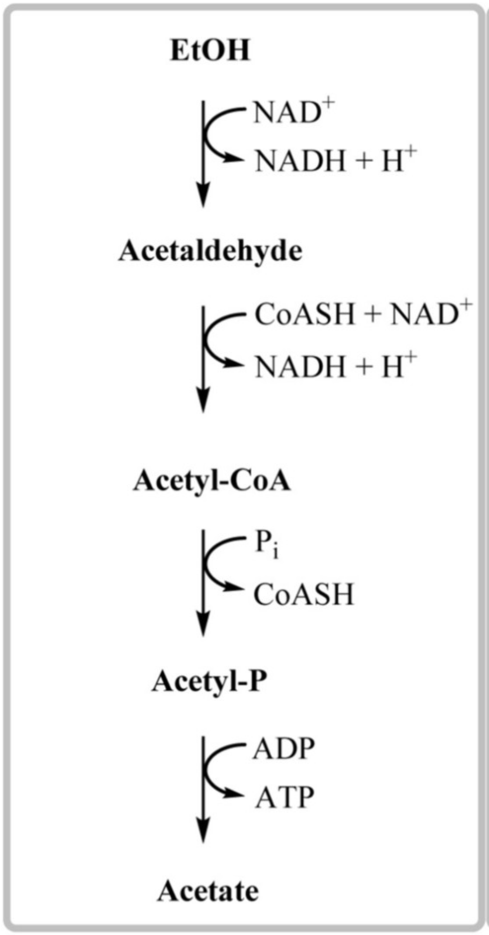
Some cellular mechanisms enable the conservation of a part of the Gibbs energy that otherwise would be dissipated

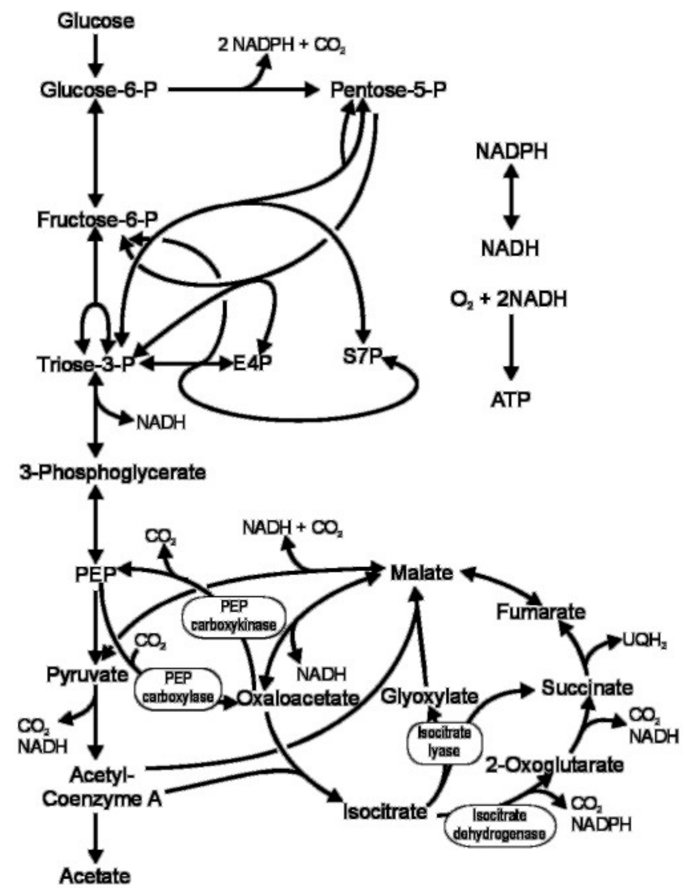


We know how some *metabolic reactions* enable the ATP generation

Ethanol + H_2O → → → → → → → → Acetate + 2 H_2

Glucose + 6 O_2 → → → → → → → → 6 CO_2 + 6 H_2O





Source: DOI 10.1074/jbc.M307968200

However, some metabolic reactions are thermodynamically unfeasible under the standard conditions

Anaerobic syntrophic bacterium

reactions	ΔrG° (kJ/mol)
Alcohol dehydrogenase	17.97
acaldDH	-21.9
Phosphotransacetylase	8.35
ACK	-13.74
Hydrogenase	29.52

Aerobic glucose oxidation

reactions	ΔrG° (kJ/mol)
Fructose-1,6-aldolase	23.98
6-phosphogluconate dehydrogenase	10.16
Aconitase	7.14
Triose-phosphate isomerase	5.63
Isocitrate dehydrogenase	5.59
Phosphoglucomutase	4.53
Phosglucose isomerase	2.64
Adenylate kinase	0.26
GAPDH	-0.97
TALA	-0.97
RPI	-2.17
ACONTb	-2.76
RPE	-3.42
ENO	-3.77
TKT1	-3.86
G6PDH	-8.92
PEPsyn	-9.08
TKT2	-10.37
GLNS	-16.93
PGK	-19.42
PFK	-20.68
PGL	-27.45
GlutamateDH	-31.11
PDH	-34.08
CS	-40.52
PEPC	-41.73
GLUCpts	-45.27
pntAB	-63.61
NADH_ETC	-256.79

Which metabolite concentrations enable the operation of these sets of reactions?

The metabolite concentrations allowing a thermodynamic feasible operation of the metabolic networks can be determined by solving an **optimization problem**

eQuilibrator

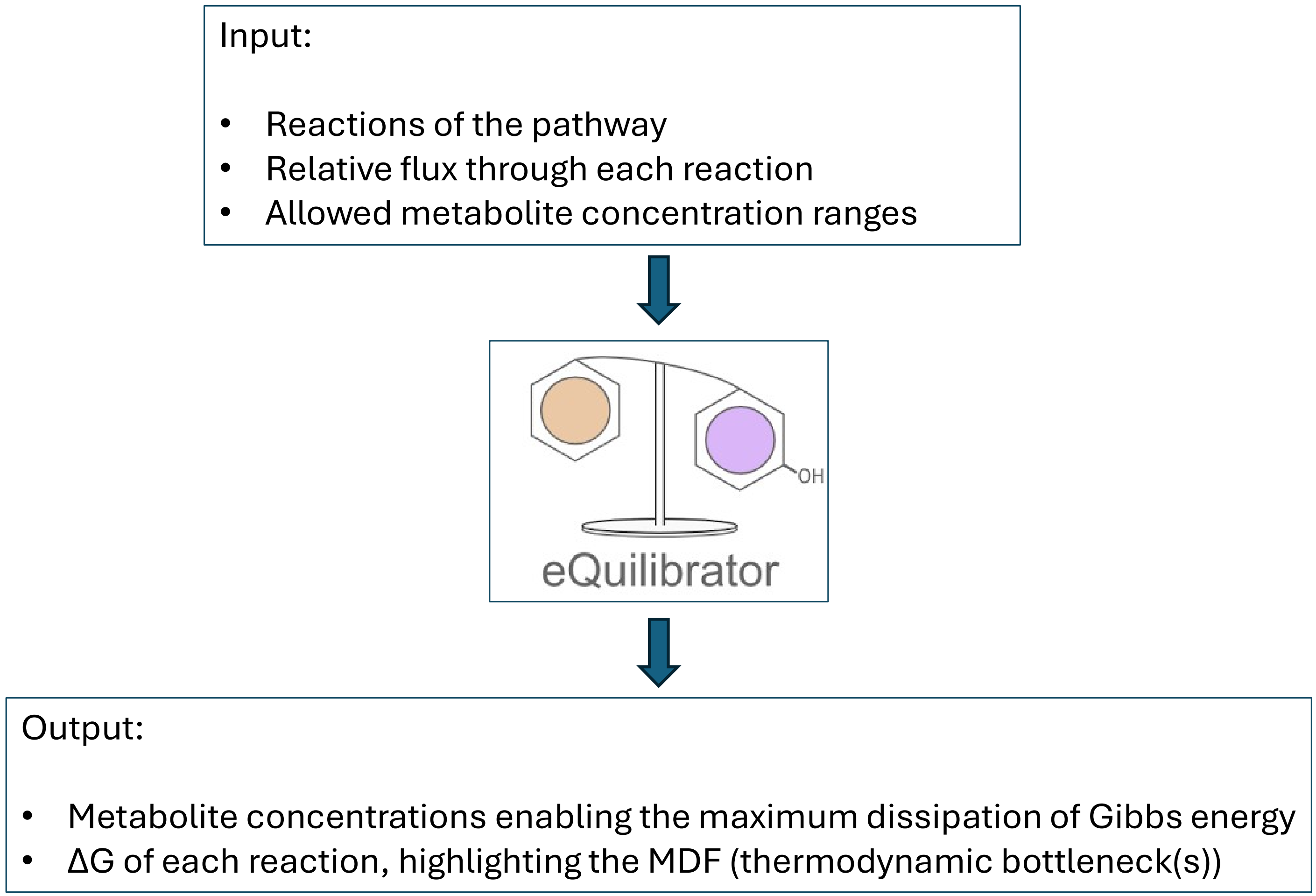
https://equilibrator.weizmann.ac.il/

The algorithms contained in eQuilibrator calculate:

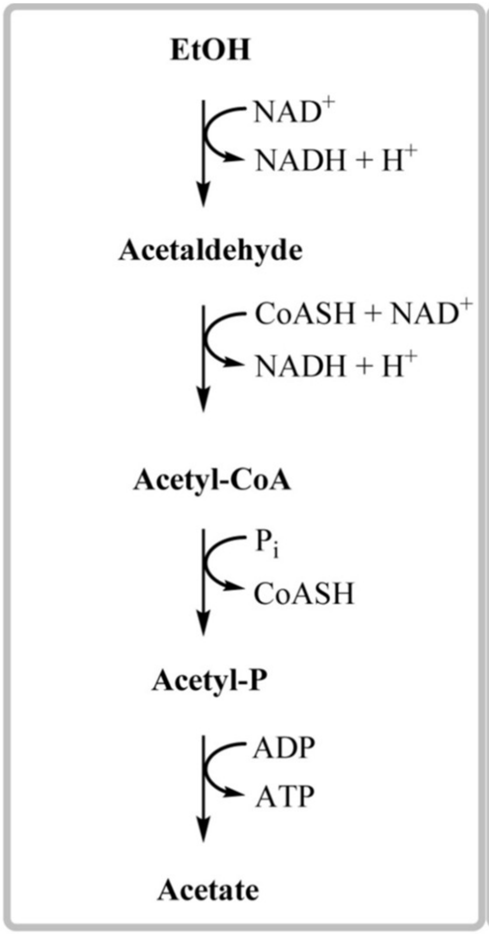
1) Metabolite concentrations maximizing -ΔrG.
2) ΔrG values associated with each reaction.
3) Determine **"MDF".**

MDF = -ΔrG of the reaction(s) dissipating the smallest amount of Gibbs energy.

MDF stands for **M**in-Max **D**riving **F**orce.


Input:
• Reactions of the pathway
• Relative flux through each reaction
• Allowed metabolite concentration ranges
eQuilibrator
Output:
• Metabolite concentrations enabling the maximum dissipation of Gibbs energy
• ΔG of each reaction, highlighting the MDF (thermodynamic bottleneck(s))

INPUT



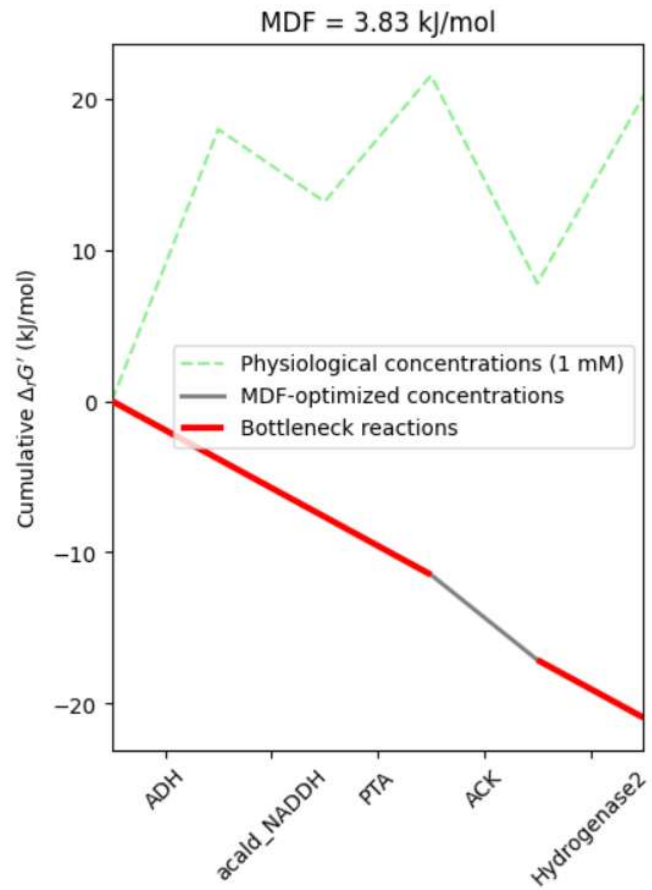
Reaction Formula	Relative Flux	Reaction Name
ethanol + NAD <=> ACDH + NADH	1	ADH
ACDH + CoA + NAD <=> AcCoA + NADH	1	acald_NADDH
AcCoA + Pi <=> AcP + CoA	1	PTA
AcP + ADP <=> acetate + ATP	1	ACK
NADH <=> NAD + H2	2	Hydrogenase

Metabolite	Lower Bound (M)	Upper Bound (M)
AcCoA	0.000001	0.01
acetaldehyde	0.000001	0.01
acetate	0.000001	0.01
AcP	0.000001	0.01
CO2	0.000001	0.01
CoA	0.000001	0.01
ethanol	0.000001	0.01
H2	0.000001	0.008
ADP	0.0002	0.0002
NADH	0.0007	0.0007
NAD	0.001	0.001
ATP	0.002	0.002
H2O	1	1
Pi	0.000001	0.03

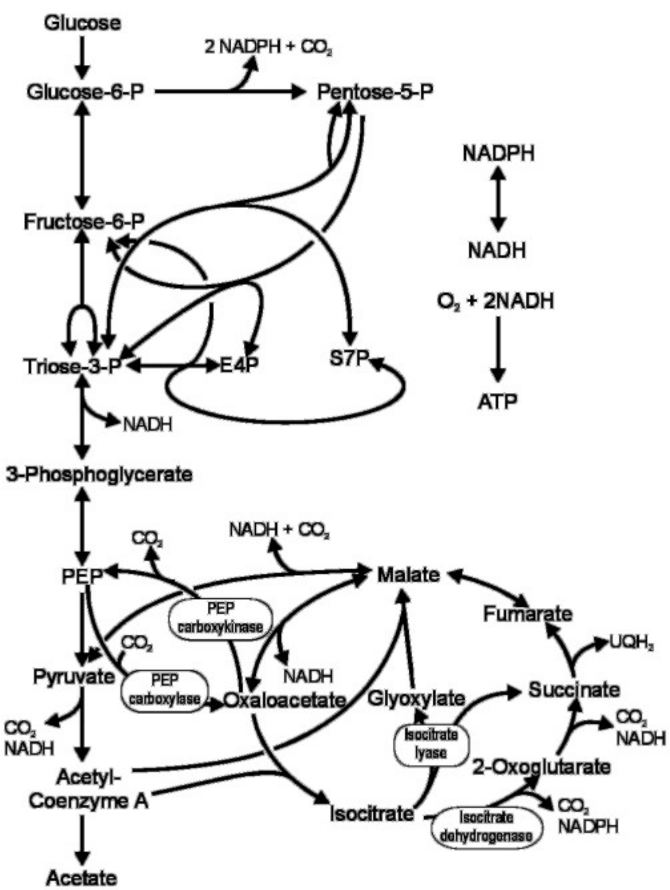
OUTPUT

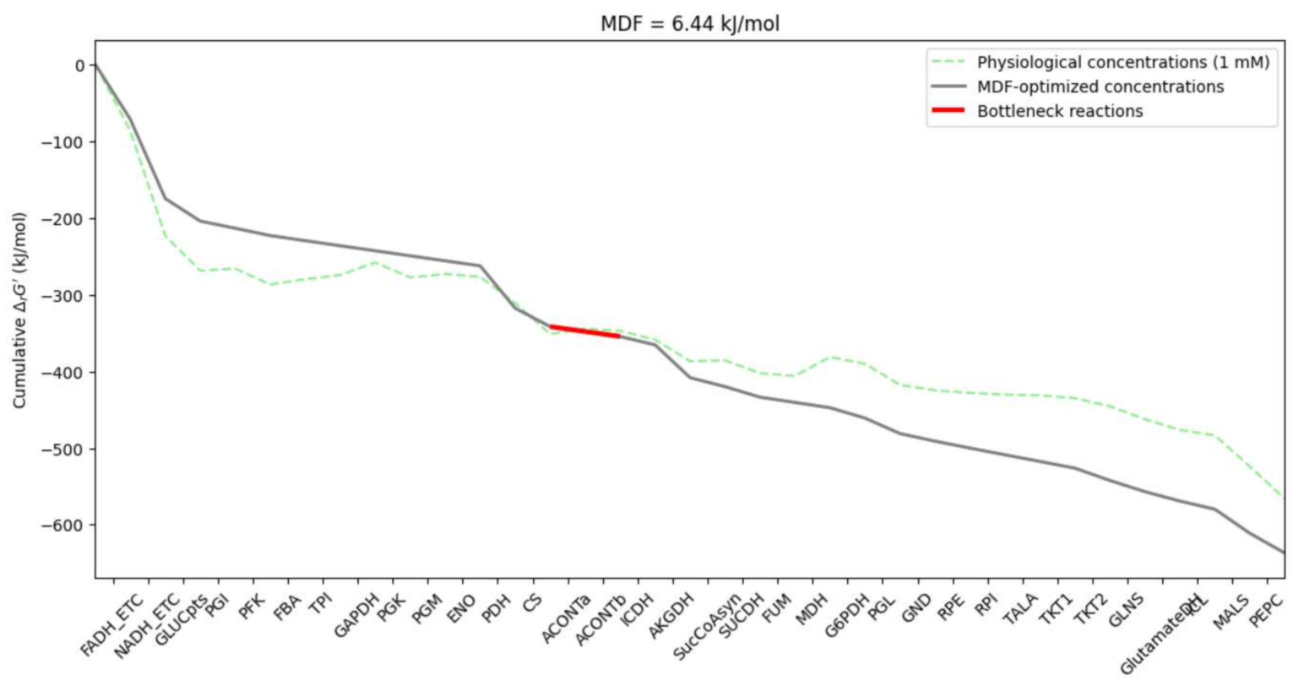
Compound	Concentration (M)
H2O	1.000000
ATP	0.002000
ADP	0.000200
NAD	0.001000
Pi	0.030000
NADH	0.000700
CoA	0.001481
AcCoA	0.000007
acetate	0.000003
ACDH	0.000002
H2	0.000001
AcP	0.000001
ethanol	0.010000

reactions	Optimized ΔrG (kJ/mol)
ADH	**-3.83**
acald_NADDH	**-3.83**
PTA	**-3.83**
ACK	-5.63
Hydrogenase2	**-3.83**

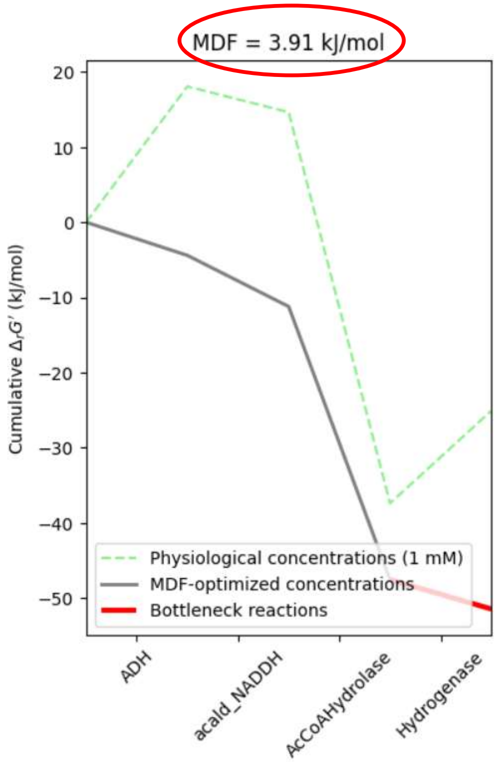


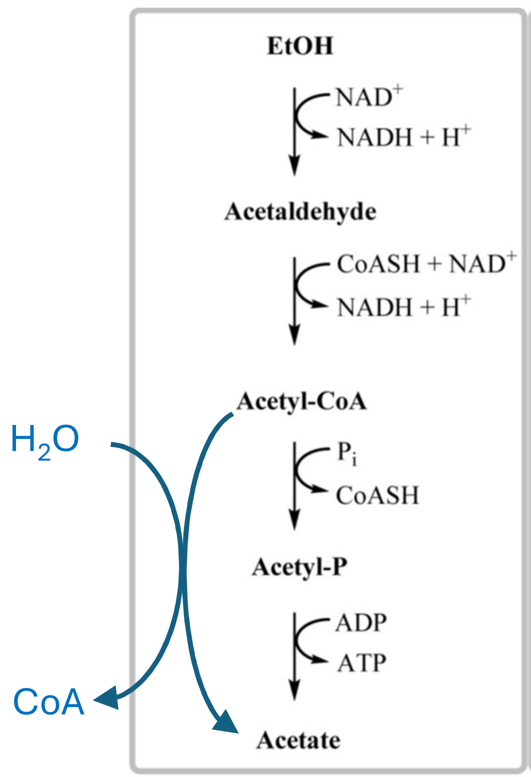
Analysis of the aerobic oxidation of glucose using eQuilibrator

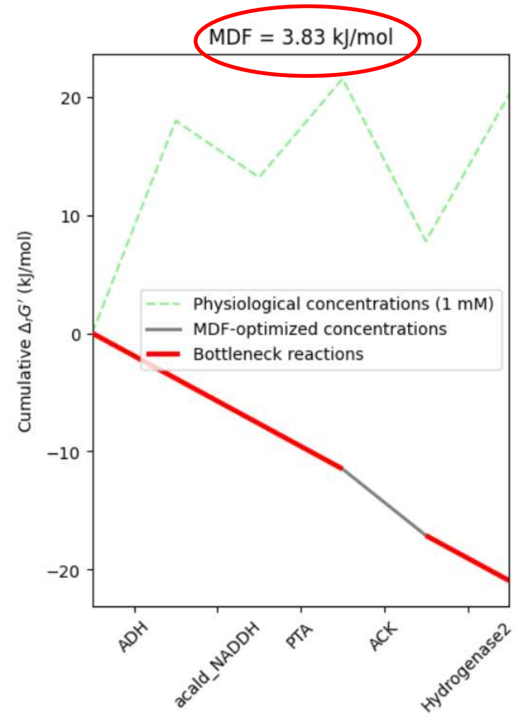




How does energy conservation impact the operation of metabolic pathways?

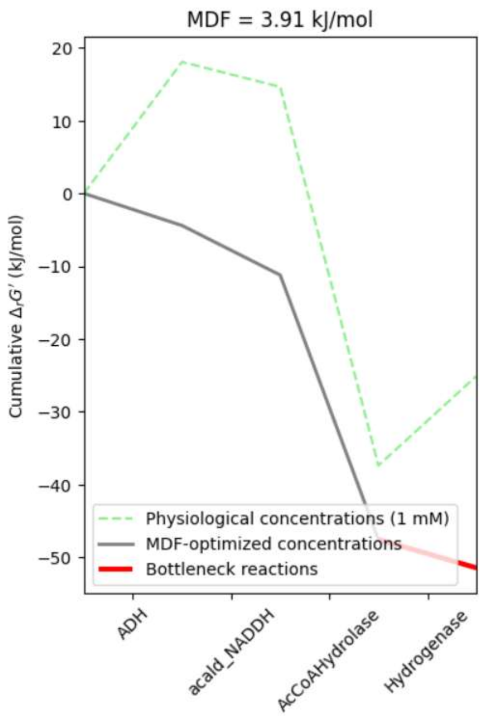


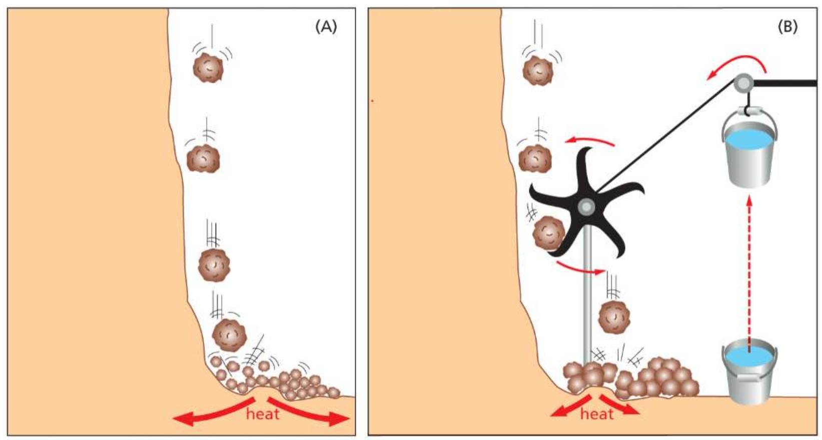


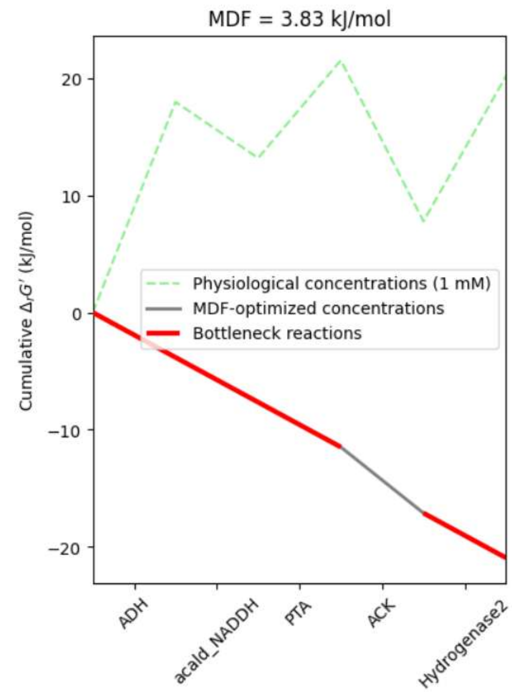


Why does ATP conservation decrease the MDF?

Energy conservation decreases the Gibbs energy that can be dissipated



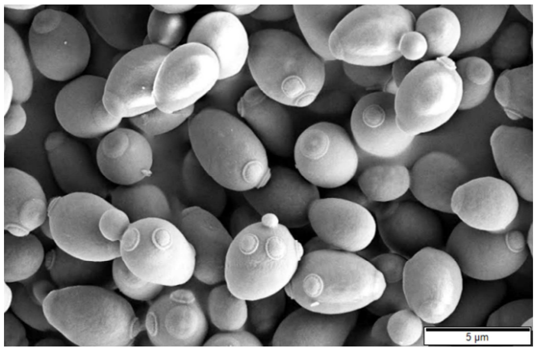




H_2O + Ethanol <=> acetate + 2 H_2

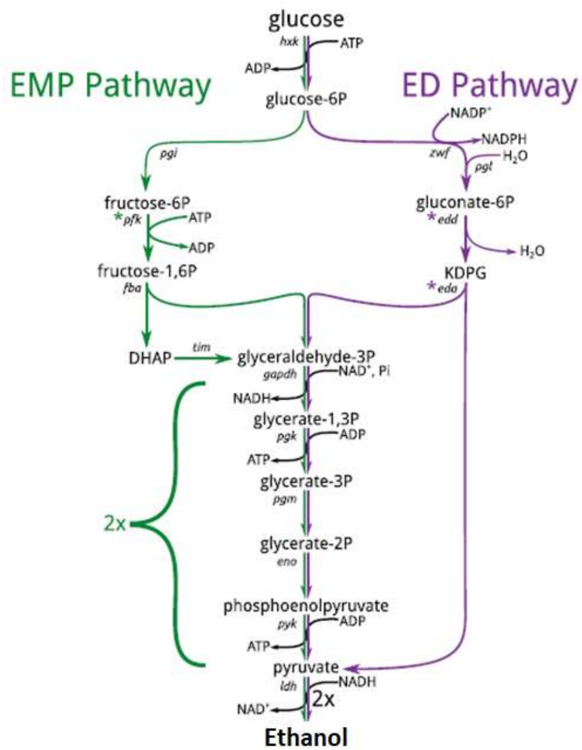
ADP + Pi + Ethanol <=> ATP + acetate + 2 H_2

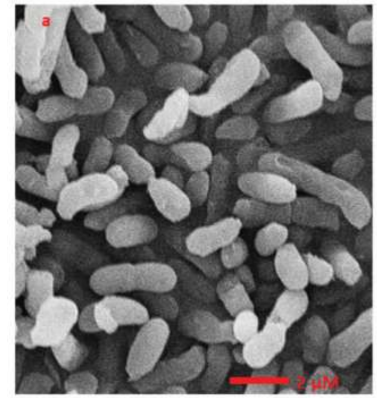
Quiz: Which of these pathways should have a larger MDF?



Saccharomyces cerevisiae

2 ADP + 2 Pi + Glucose = 2 H_2O + 2 ATP + 2 CO_2 + 2 Ethanol

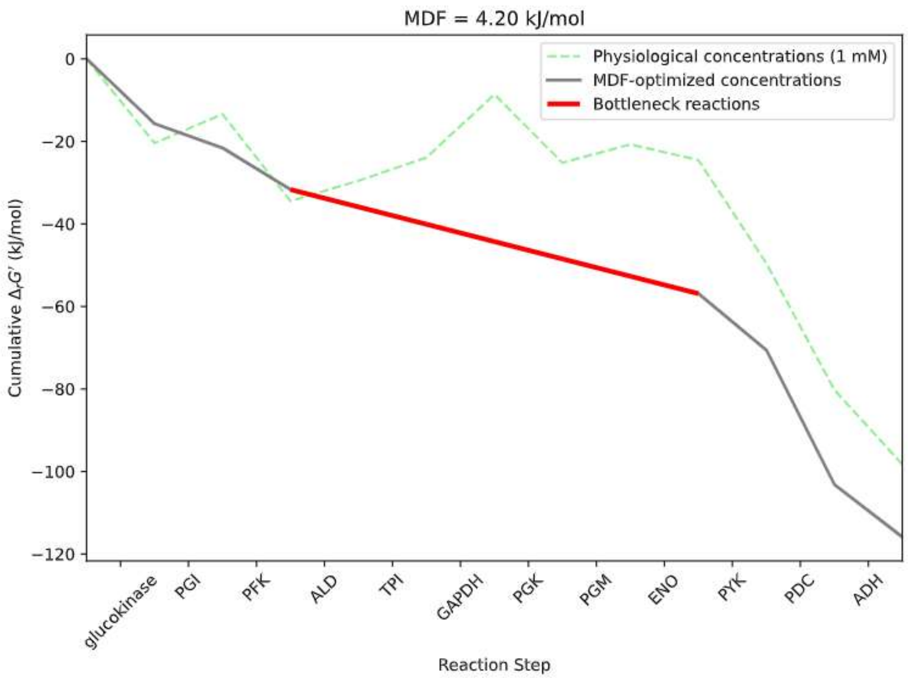




Zymomonas mobilis

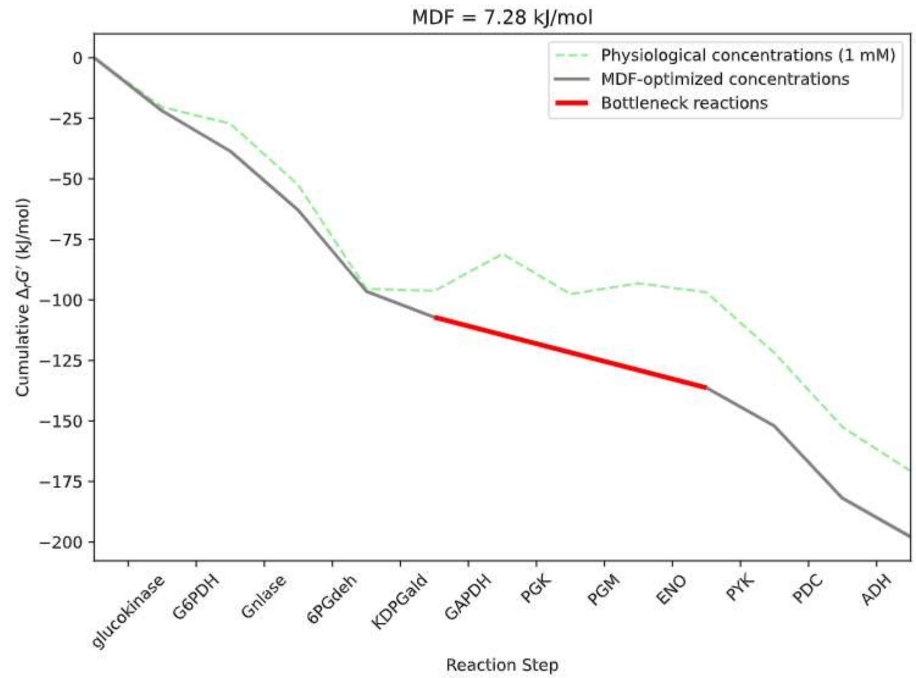
ADP + Pi + Glucose = H_2O + ATP + 2 CO_2 + 2 Ethanol

EMP



2 ADP + 2 Pi + Glucose = 2 H_2O + 2 ATP + 2 CO_2 + 2 Ethanol

ED



ADP + Pi + Glucose = H_2O + 1 ATP + 2 CO_2 + 2 Ethanol

Quiz: Which of these pathways should have a larger MDF?

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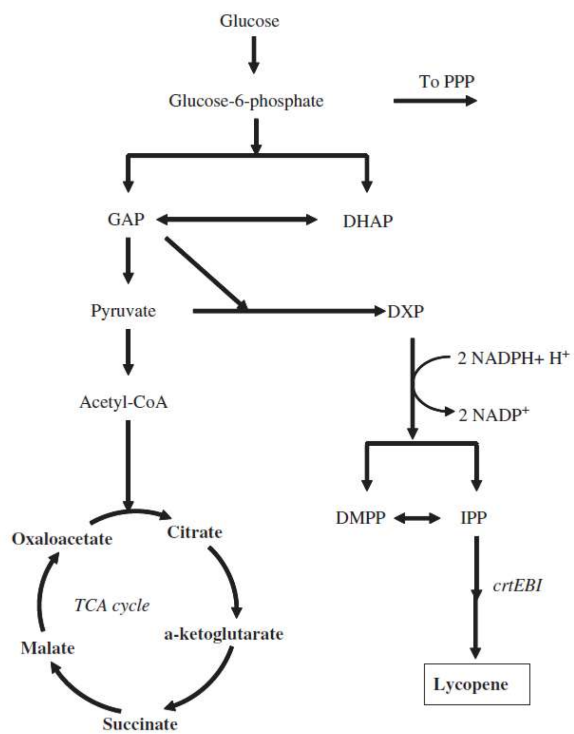
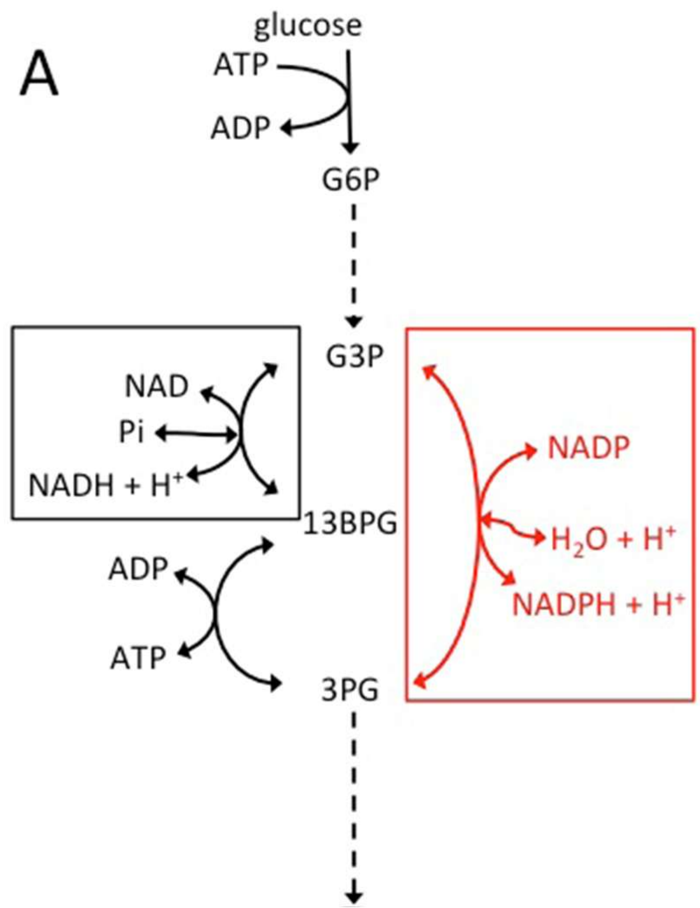
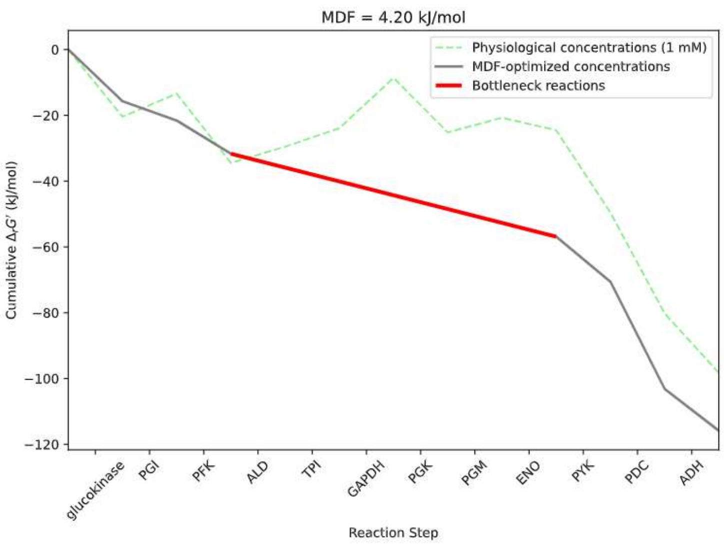
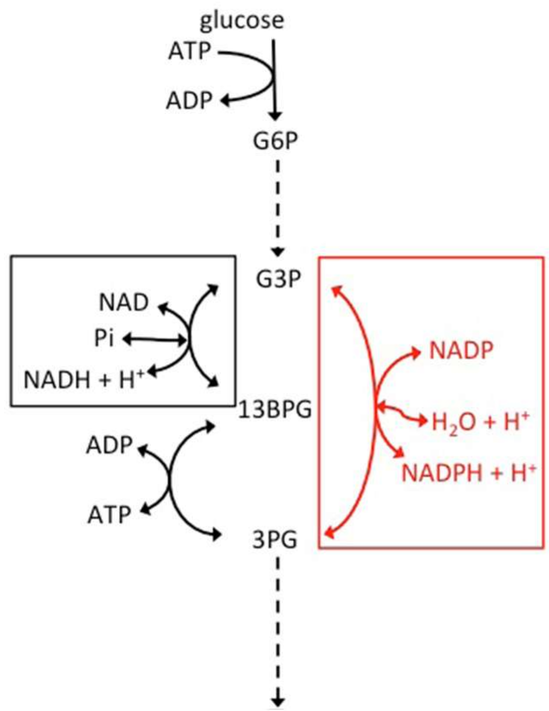
Replacing *Escherichia coli* NAD-dependent glyceraldehyde 3-phosphate dehydrogenase (GAPDH) with a NADP-dependent enzyme from *Clostridium acetobutylicum* facilitates NADPH dependent pathways

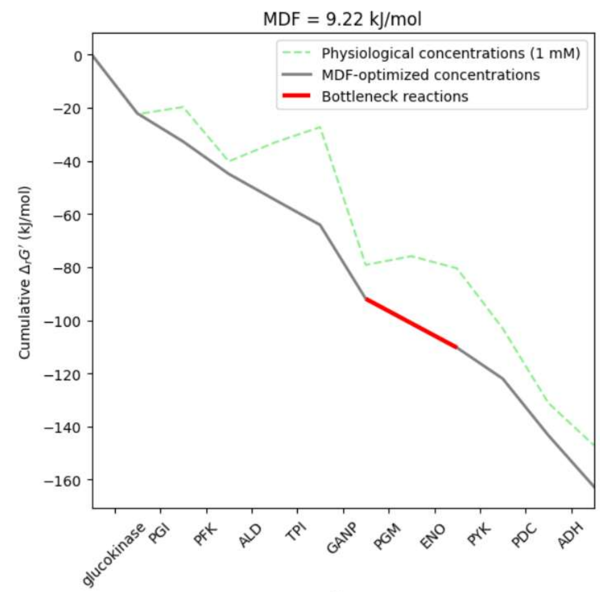
Irene Martínez [a], Jiangfeng Zhu [a], Henry Lin [a,1], George N. Bennett [b], Ka-Yiu San [a,*]











2 ADP + 2 Pi + Glucose = 2 H_2O + 2 ATP + 2 CO_2 + 2 Ethanol

Glucose = 2 CO_2 + 2 Ethanol

What is the impact of having a larger thermodynamic driving force?

$$v = \frac{\frac{k_{cat}^{f}}{K_s} * E * S - \frac{k_{cat}^{r}}{K_p} * E * P}{1 + \frac{S}{K_s} + \frac{P}{K_p}} \quad \longrightarrow \quad E = \frac{v}{k_{cat}^{f} * \left(1 - \left(\frac{1}{K_{eq}} * \frac{P}{S}\right)\right) * \left(\frac{\frac{S}{K_s}}{1 + \frac{S}{K_S} + \frac{P}{K_p}}\right)}$$

Catalytic power

"distance" from thermodynamic equilibrium

Level of saturation

The amount of enzyme required to sustain a given metabolic flux (enzyme cost) depends on the kinetic properties of the enzyme and on how far from the thermodynamic equilibrium the reaction is.

Enzyme cost for sustaining a metabolic flux of 0.148 M/s, through the triose-phosphate isomerase, at different thermodynamic driving forces

DHAP (μM)	**G3P (μM)**	**ΔrG (kJ/mol)**	***v* (M/s)**	**required Enzyme to sustain the flux (μM of enzyme /Lcyt)**
2898	22	-5.89	0.148	0.030
2188	19	-5.61	0.148	0.034
1477	15	-5.14	0.148	0.042
767	12	-4.13	0.148	0.067

$$E = \frac{v}{k_{cat}^{f} * \left(1 - \left(\frac{1}{K_{eq}} * \frac{G3P}{DHAP}\right)\right) * \left(\frac{\frac{DHAP}{K_{M\ DHAP}}}{1 + \frac{DHAP}{K_{M\ DHAP}} + \frac{G3P}{K_{M\ G3P}}}\right)}$$

The closer to zero is ΔG, the more enzyme is required to sustain the same flux

Why?

The closer to zero is ΔG, the larger the extension of the backward flux and the larger the enzyme cost

Flux-Force relationship

The backward flux increases exponentially

$$Flux^{backward} = Flux^{forward} * e^{\frac{\Delta_r G}{RT}}$$

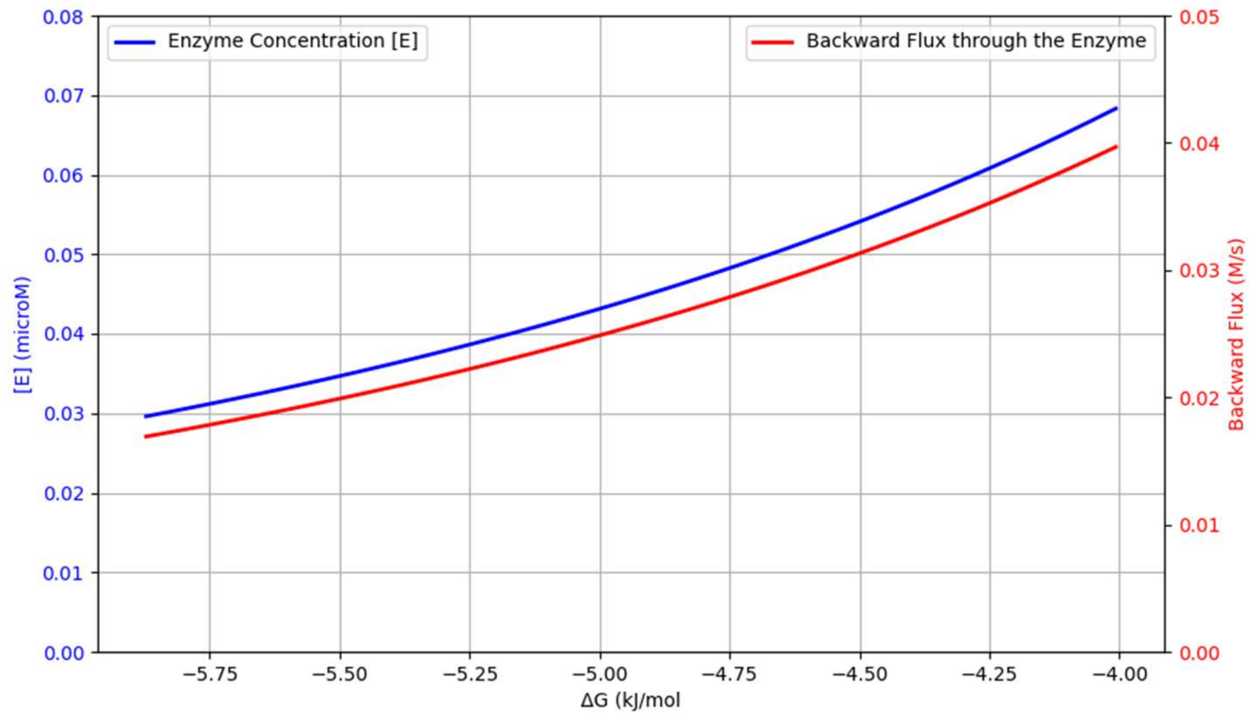
$$E = \frac{v}{k_{cat}^{f} * \left(1 - \left(\frac{1}{K_{eq}} * \frac{P}{S}\right)\right) * \left(\frac{\frac{S}{K_s}}{1 + \frac{S}{K_S} + \frac{P}{K_p}}\right)}$$

As ΔG<0 becomes closer to 0, more enzyme molecules are “busy” catalyzing the backward flux

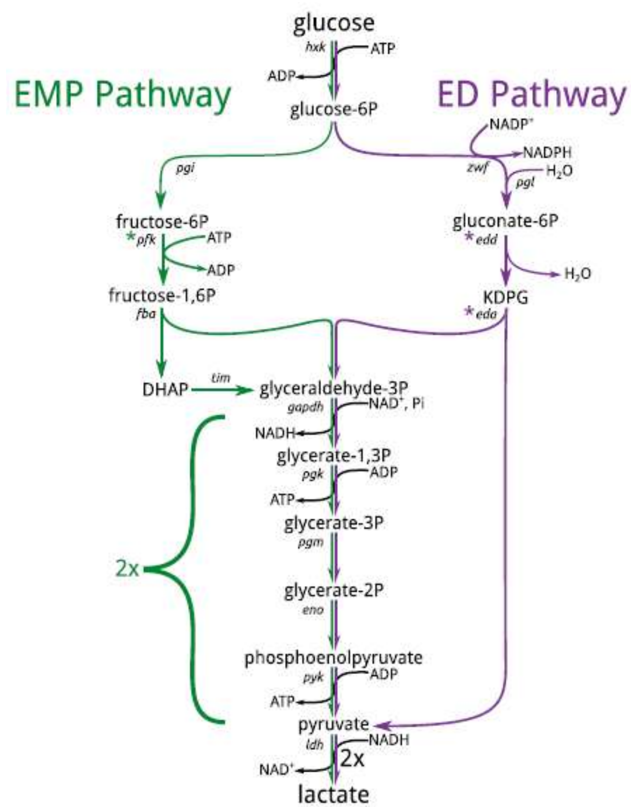
$$Flux^{backward} = Flux^{forward} * e^{\frac{\Delta_r G}{RT}}$$

$$E = \frac{v}{k_{cat}^{f} * \left(1 - \left(\frac{1}{K_{eq}} * \frac{P}{S}\right)\right) * \left(\frac{\frac{S}{K_s}}{1 + \frac{S}{K_S} + \frac{P}{K_p}}\right)}$$

Representation adapted to triose-phosphate isomerase

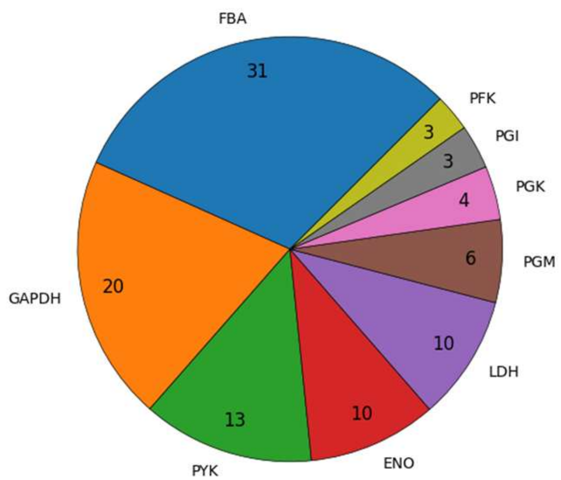


Quiz: which pathway should require more enzyme concentration to sustain the same metabolic flux?

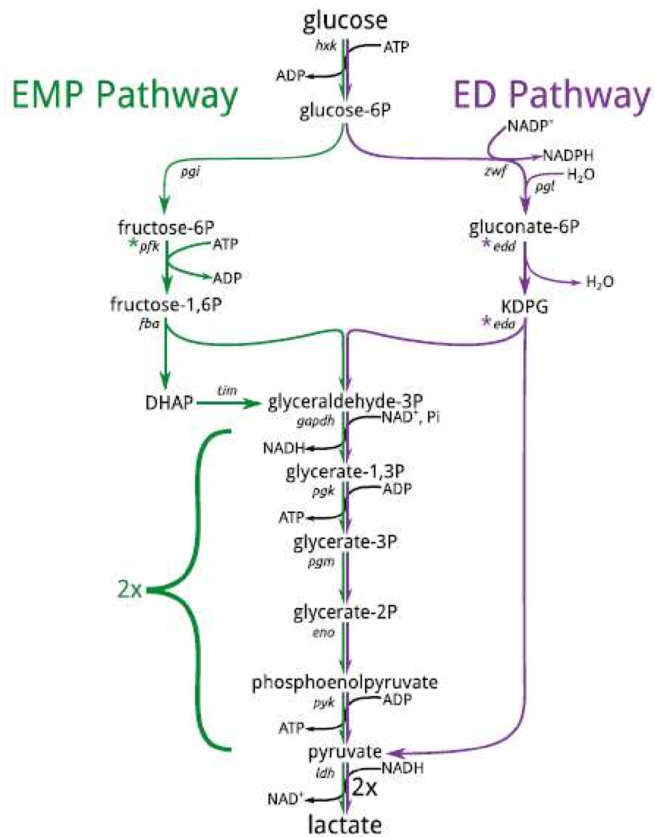


There is a trade-off between energy conservation and enzyme cost

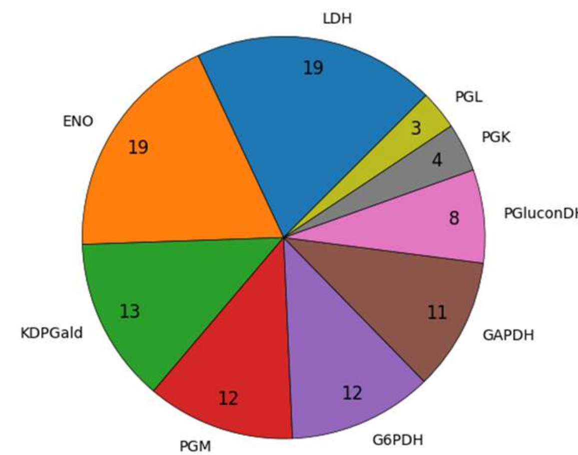
2 ATP/Glucose



6.6 g/L

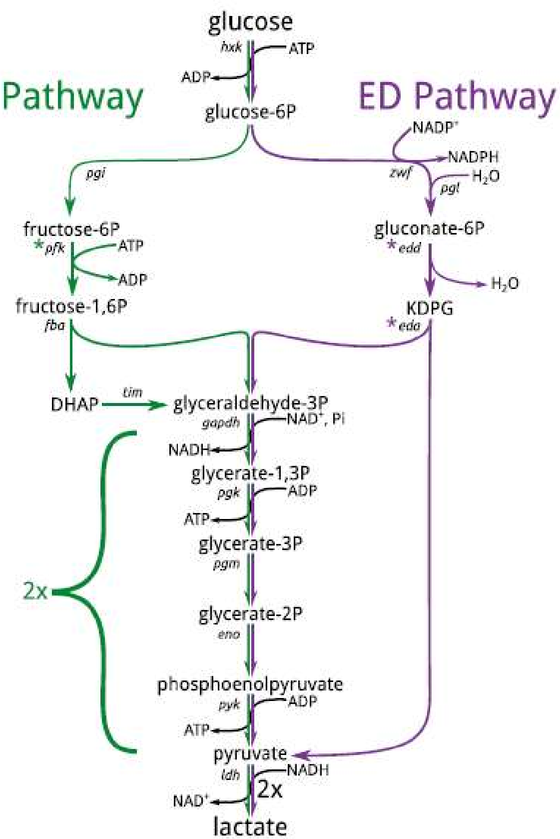


1 ATP/Glucose



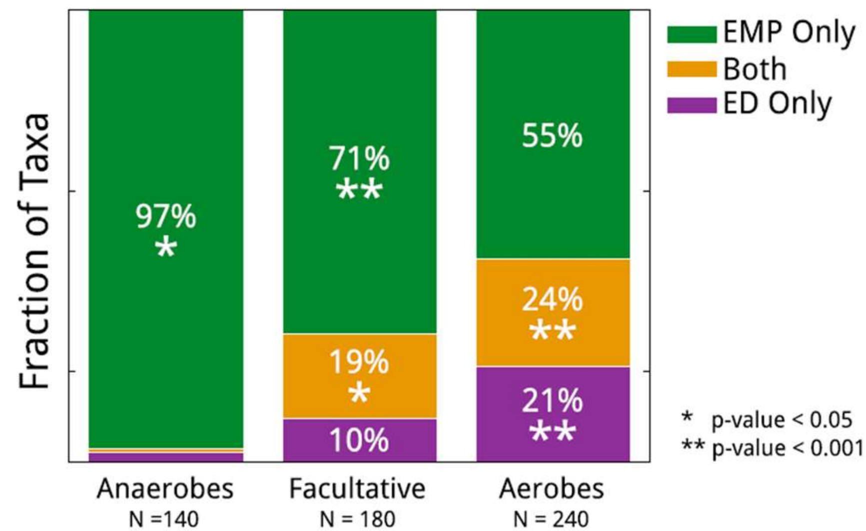
1.3 g/L

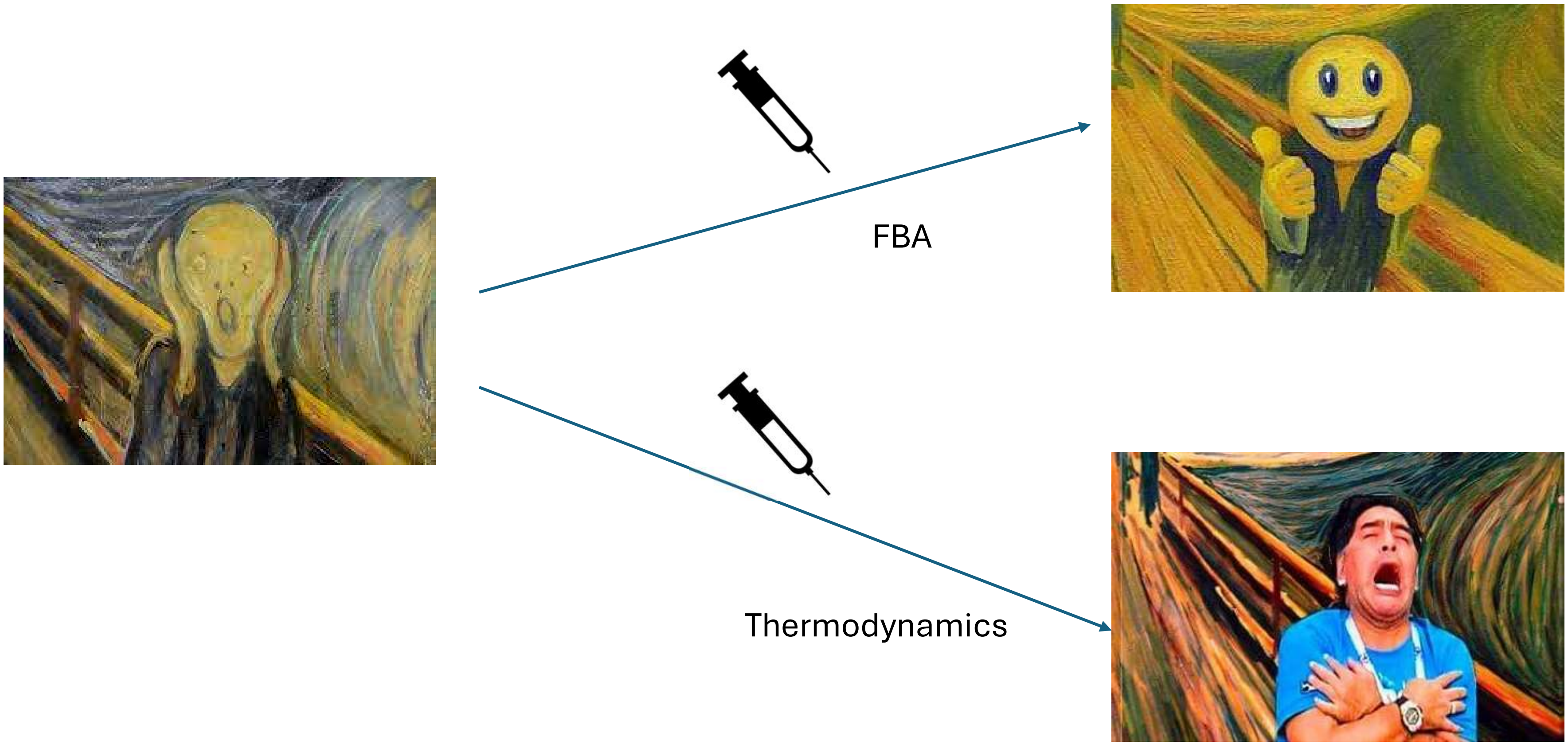
Ecological relevance of a thermodynamic analysis: the Entner-Doudoroff pathway is more frequent in aerobes



2 ATP/Glucose

1 ATP/Glucose




FBA
Thermodynamics

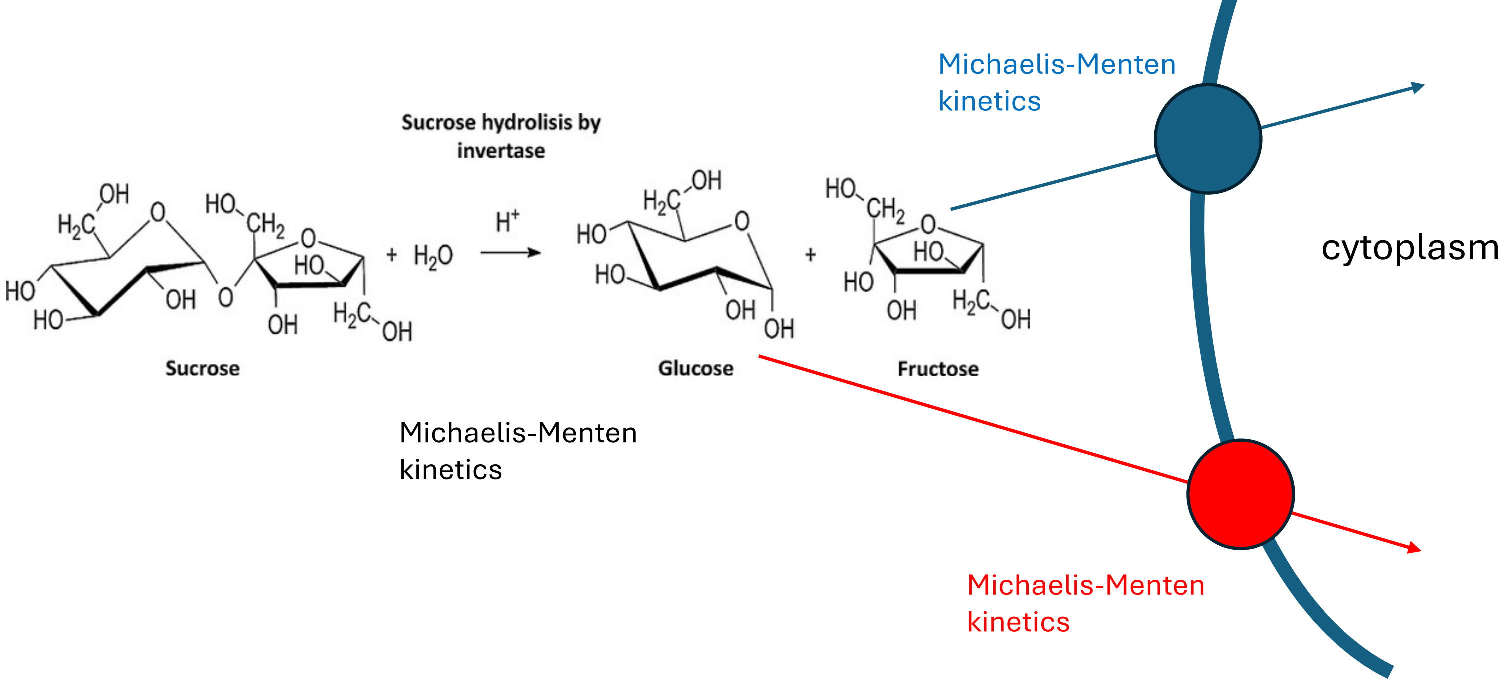
Bioethanol in Brazil is mainly produced in open fermentation

In open fermentations:

- Yeast populations are dynamic.
- Different bacteria are present and influence the ethanol production.
- Some bacteria have negative effects on the ethanol production, but some bacteria have a positive effect.

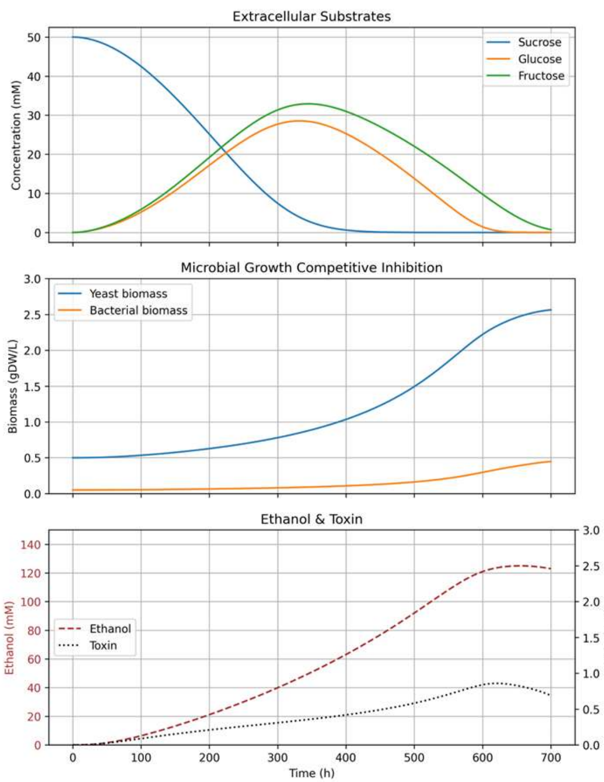
Yeast populations are dynamic

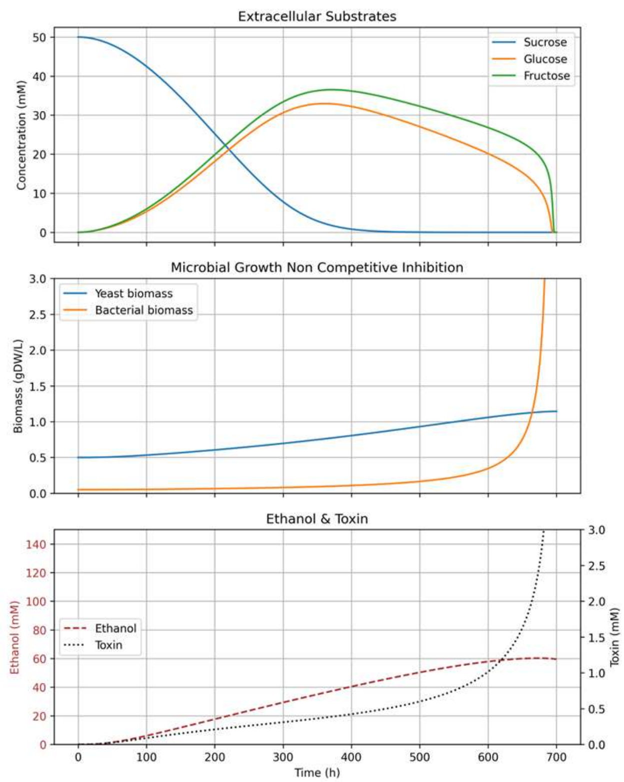
Different yeast strains can have different invertases and different transporters

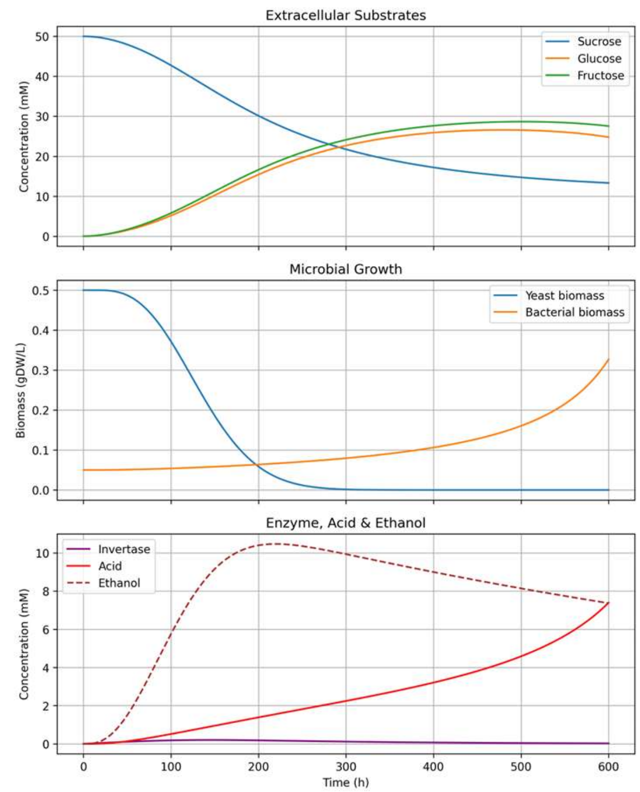


Different bacteria are present and influence the ethanol production.

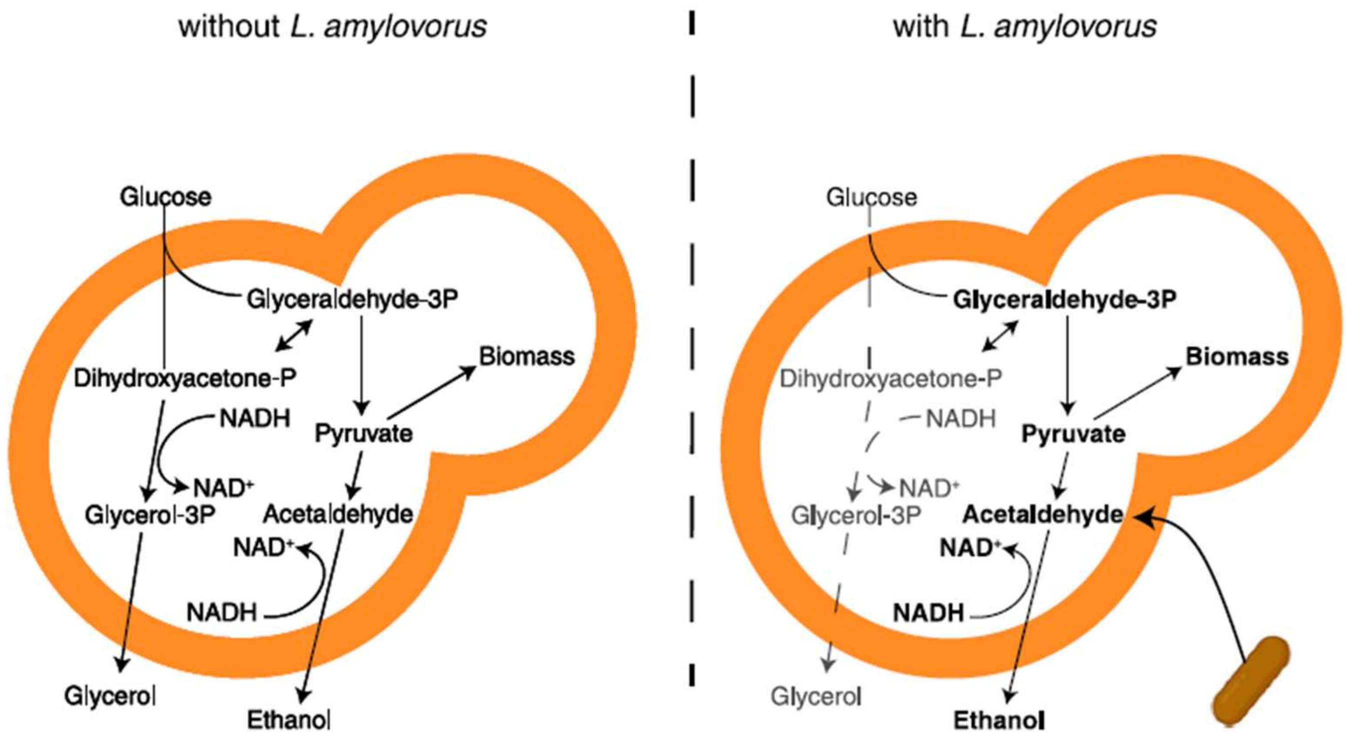
Based on simple kinetic equations, we can represent interactions among organisms







Some bacteria have negative effects on the ethanol production, but some bacteria have a positive effect



Conclusions

- The kinetic parameters of the enzymes are linked through the equilibrium constants
- Thermodynamics helps us to calculate how much ATP can be produced in the metabolic pathways
- Pathways are active if all their reactions are thermodynamically feasible
- Energy conservation impacts the thermodynamic driving force in a pathway
- Enzyme cost is determined by thermodynamic and kinetic factors
- There is a trade-off between energy conservation and enzyme cost (*there are no free cookies*)