Microbial Metabolism

Today

- Metabolism in Cellular Context
- Types of Microbial Metabolism (Catabolism)

What constrains microbial metabolism?

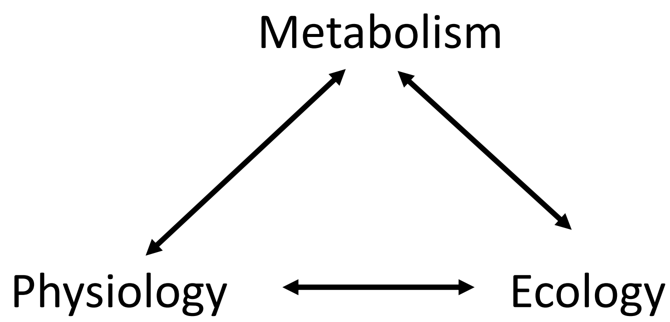
- How Microbes use Energy
- Link between Thermodynamics and Kinetics
- Speciation of Metabolic pathways
- Metabolism in Natural Selection and Isolation of Microbes

Next Thursday

Chemical constraints microbial metabolism

- Principles of Metabolic Transformations
- Patterns of Metabolism

What of metabolism is it that we want to understand?



Nothing in biology makes sense
except in light of evolution

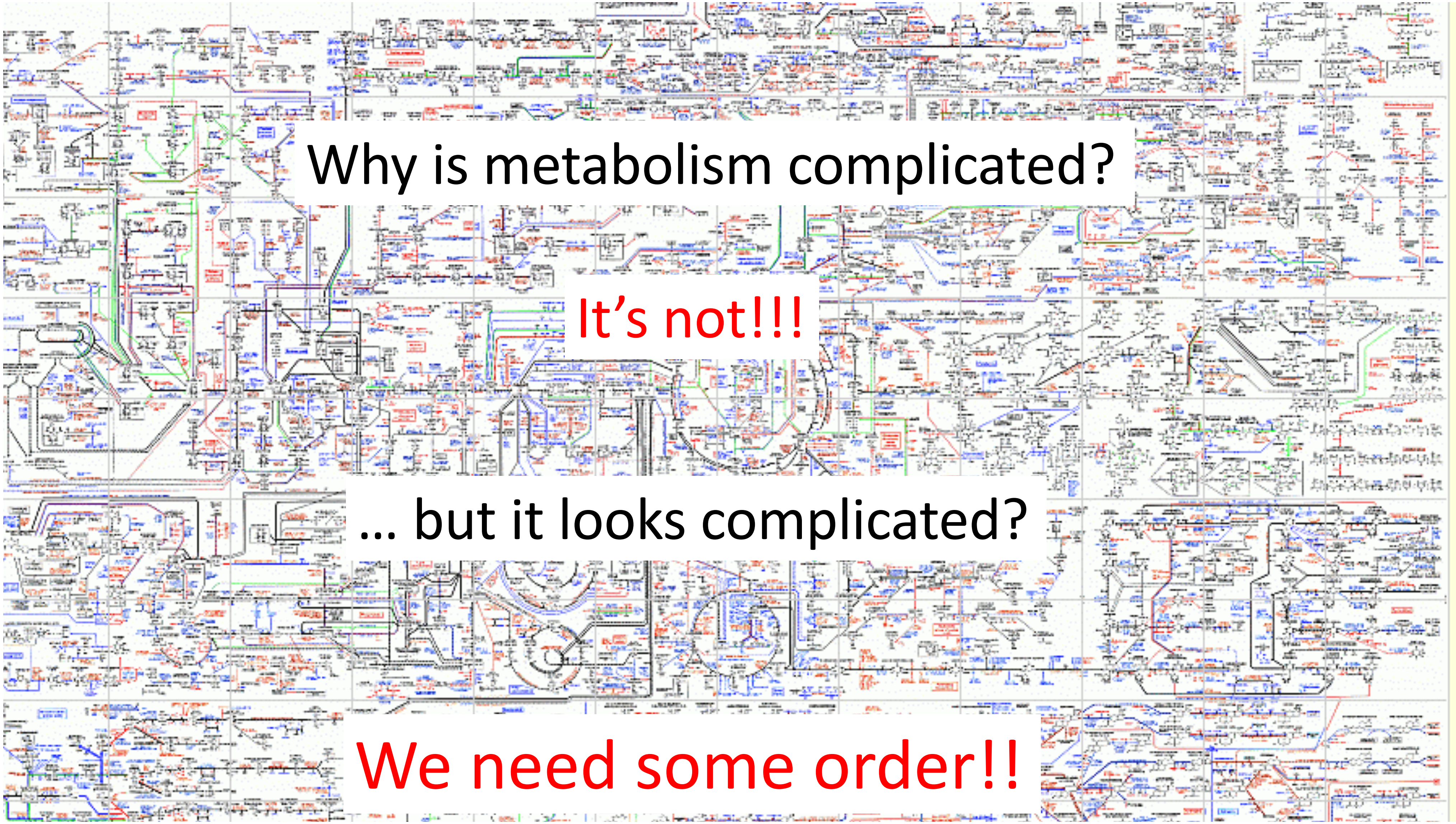
Theodosius Dobzhansky, 1973

Life is nothing but an electron
looking for a place to rest.

Albert Szent-Gyorgi

Selection is everywhere

John Roth (paraphrased)



Why is metabolism complicated?

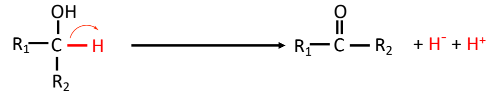
It's not!!!

… but it looks complicated?

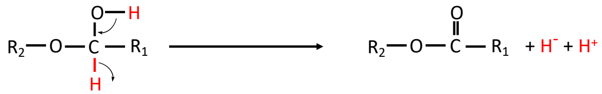
We need some order!!

Oxidations/reductions

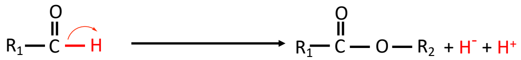
Alcohol oxidation



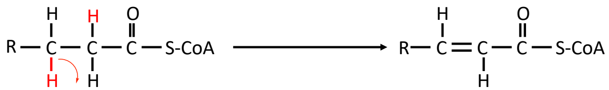
Hemiacetal oxidation



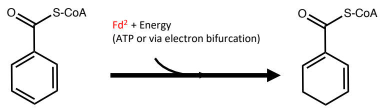
Aldehyde oxidation



Acyl-CoA oxidation



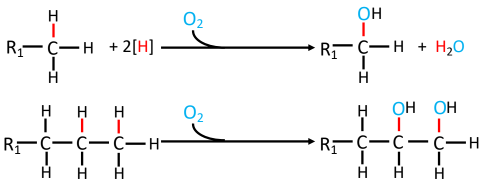
Benzoyl-CoA reduction (dearomatizing)



Methyl-CoM reduction

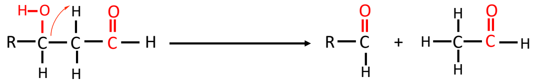
$$CH_3\text{-S-CoM} + \text{H-S-CoB} \rightleftharpoons \text{CoM-S-S-CoB} + CH_4$$

Oxygenation

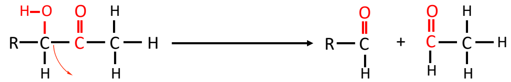


C-C cleavage/ condensations

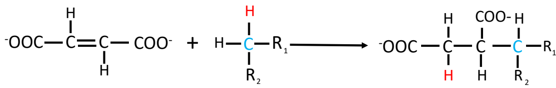
Aldol cleavage



Ketol cleavage



Fumarate addition



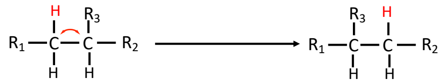
De/carbonylation

$$CO + \text{HS-CoA} + CH_3\text{-}H_4F \rightleftharpoons CH_3CO\text{-S-CoA} + H_4F$$

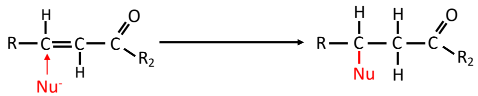
$$CO_2 + Fd^{2-} + 2H^+ \rightleftharpoons Fd + H_2O + CO$$

Auxiliary reactions

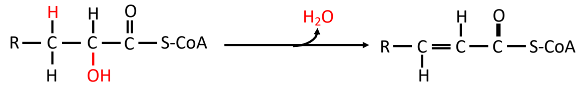
Rearrangement



Nucleophilic addition/elimination



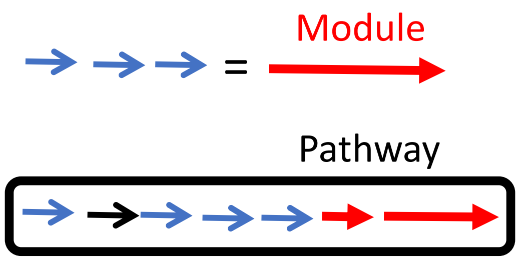
α hydroxy elimination

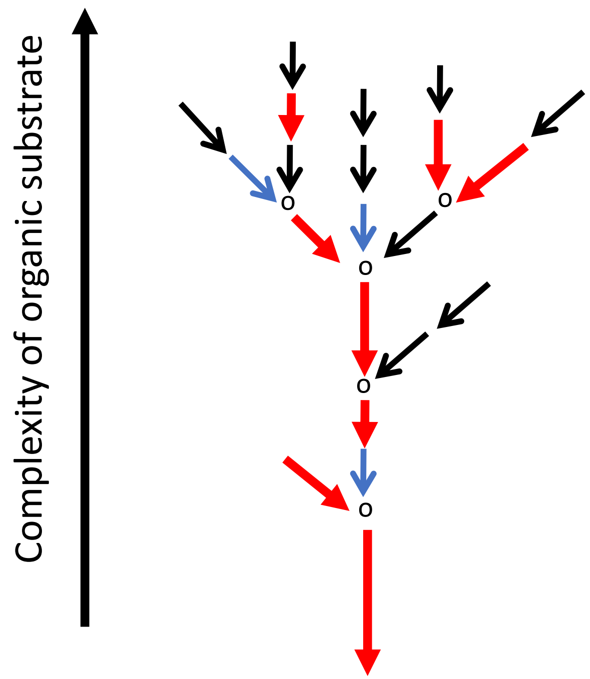


Hydrogen oxidation

$$H_2 \rightleftharpoons 2\,e^- + 2\,H^+$$

Modularity of Catabolic Pathways





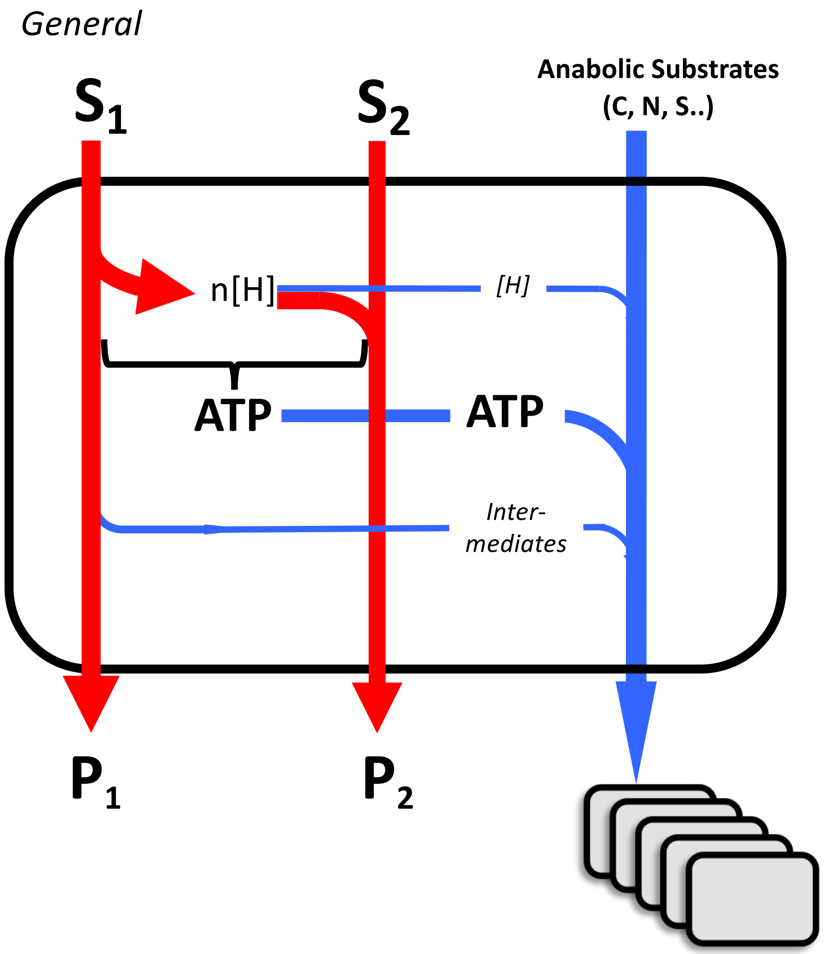
Module (more than one prototypic enzymatic reaction)

Single prototypic enzymatic reaction

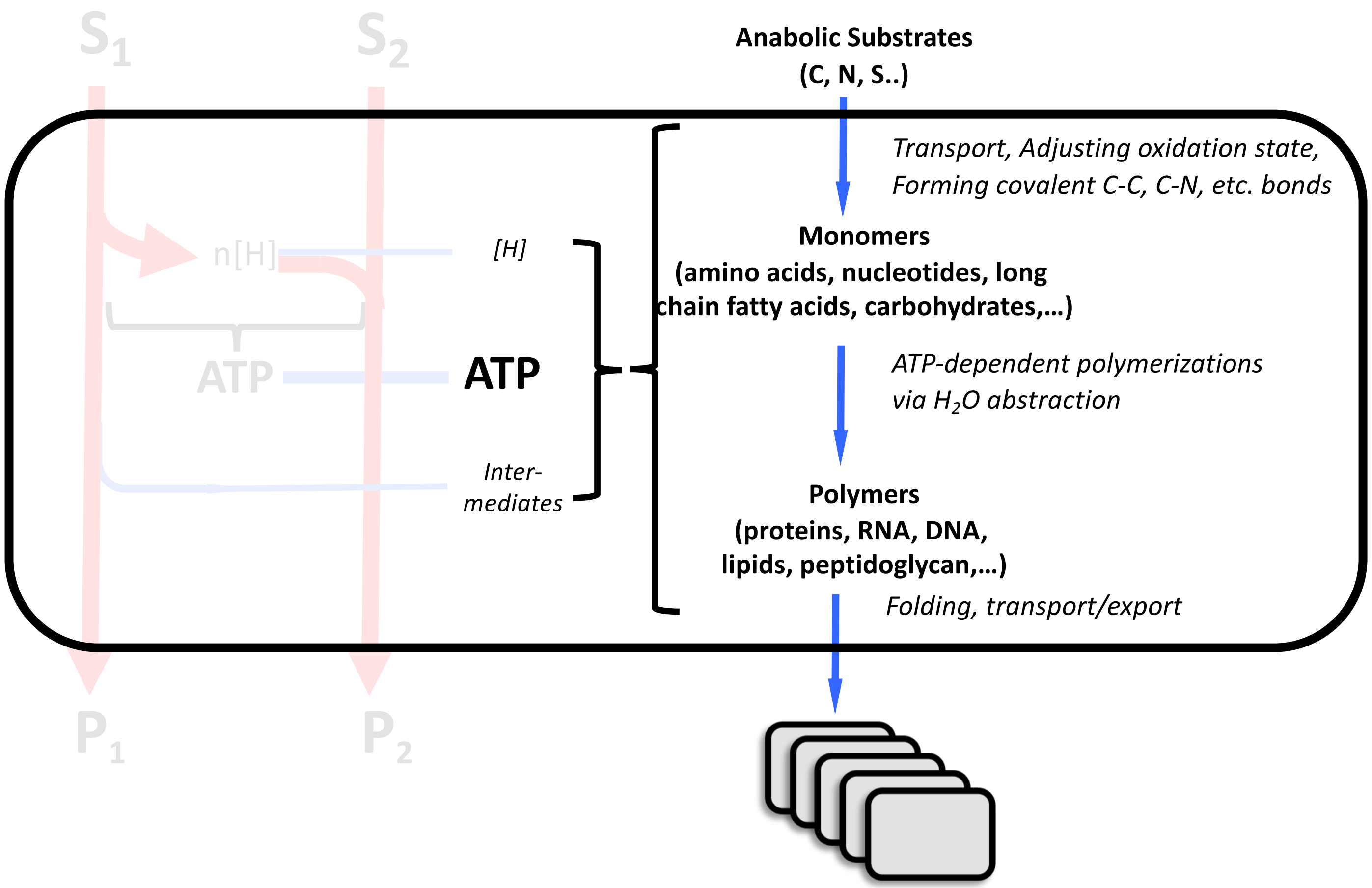
Single, less common enzymatic reaction

o Pathway node

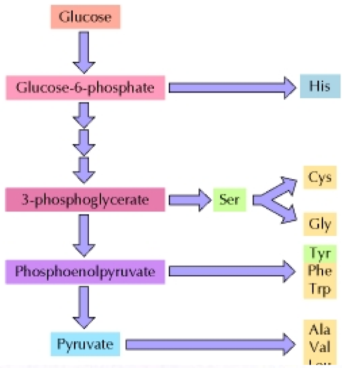
Catabolic and Anabolic Pathways

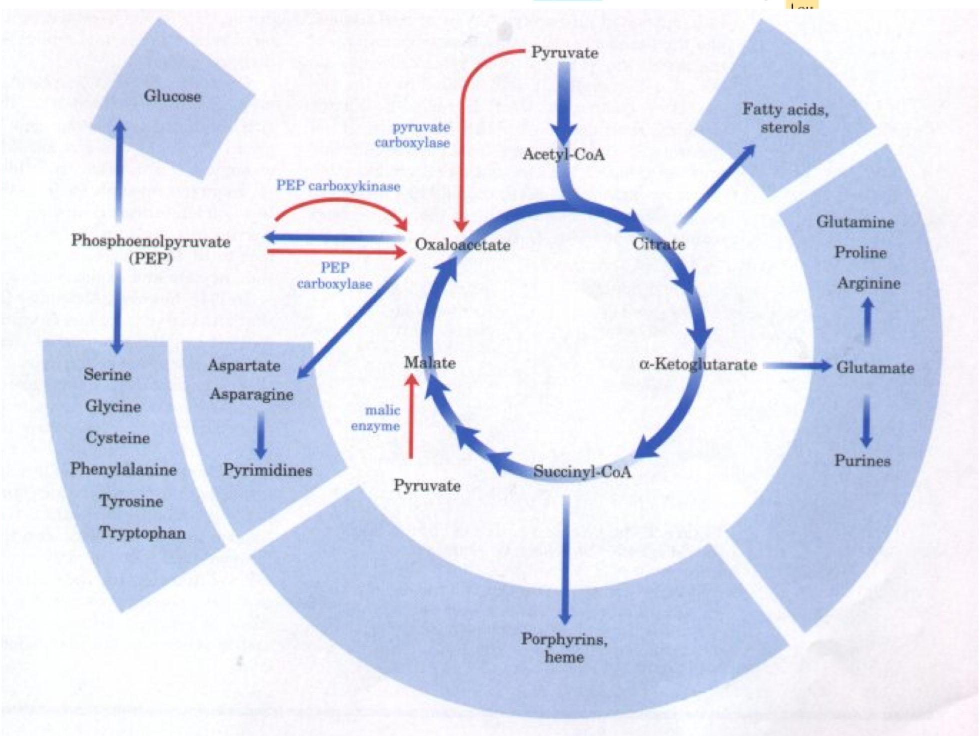


Catabolic and Anabolic Pathways

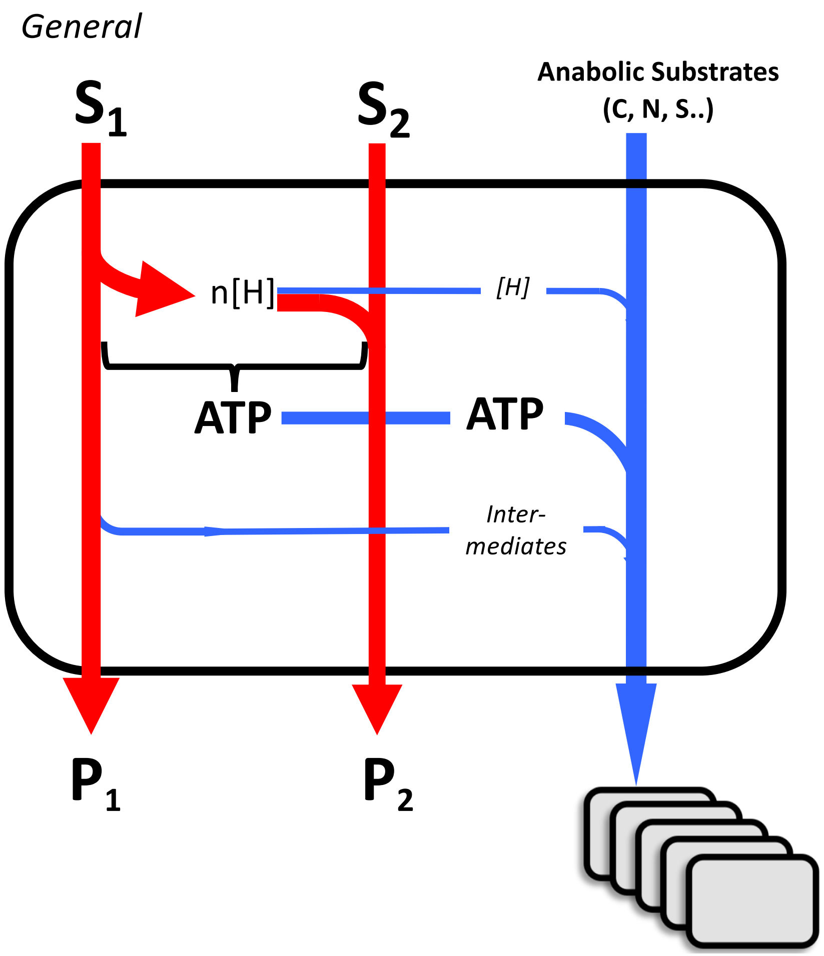


Biosynthetic precursors of amino acids, nucleic acids, and cofactors





Catabolic and Anabolic Pathways

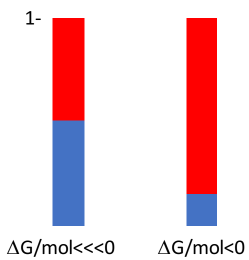


Yields:

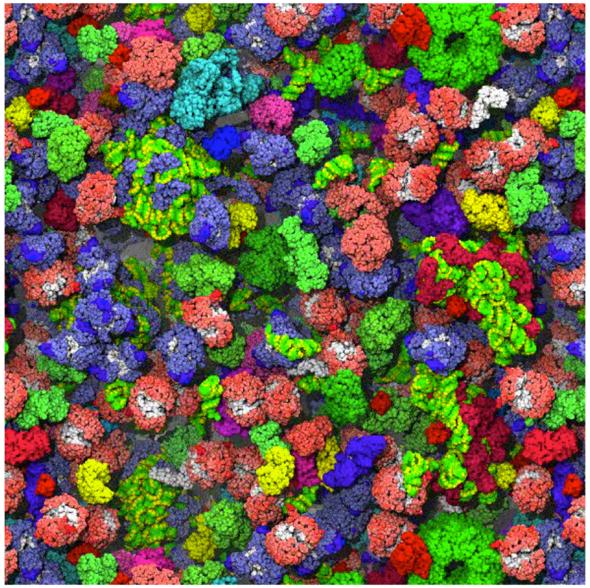
ATP/mol catabolic substrate

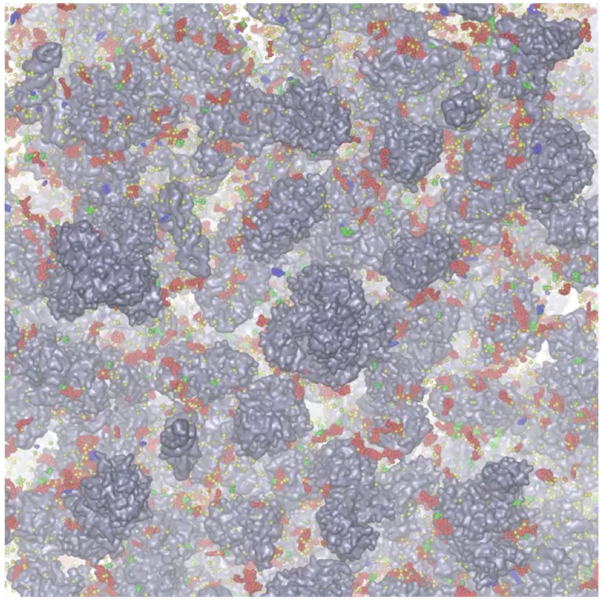
$$Y_{ATP} = 10 \frac{gdw}{mol\ ATP}$$

Fraction of carbon S in catabolism and anabolism as a function of catabolic ΔG



Molecular Crowding in a Microbial Cell





After McGuffee and Elcock 2009

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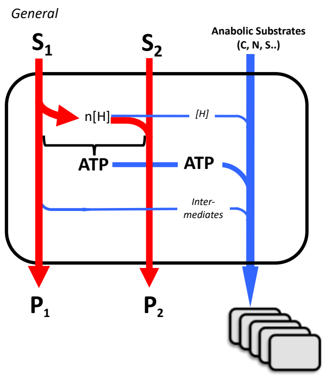
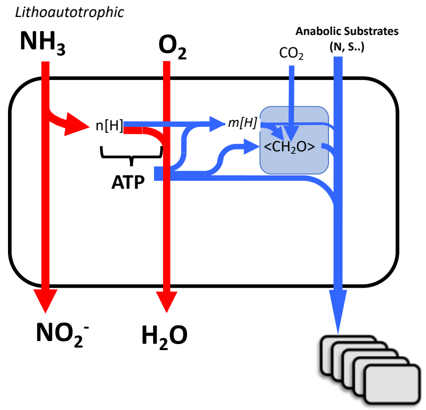
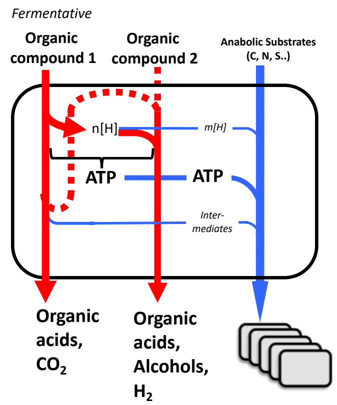
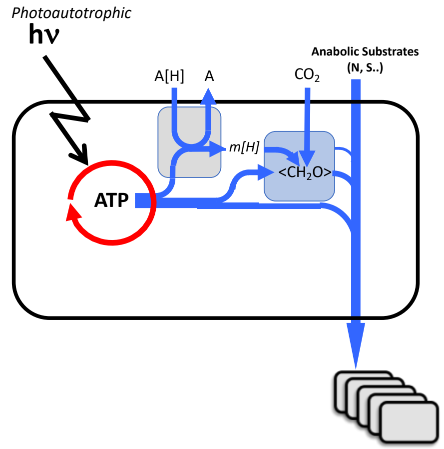
Types of Microbial Catabolism

Features and Environmental Footprints of Major Types of Microbial Catabolic and Anabolic Pathways









Metabolic Definitions of Microbes

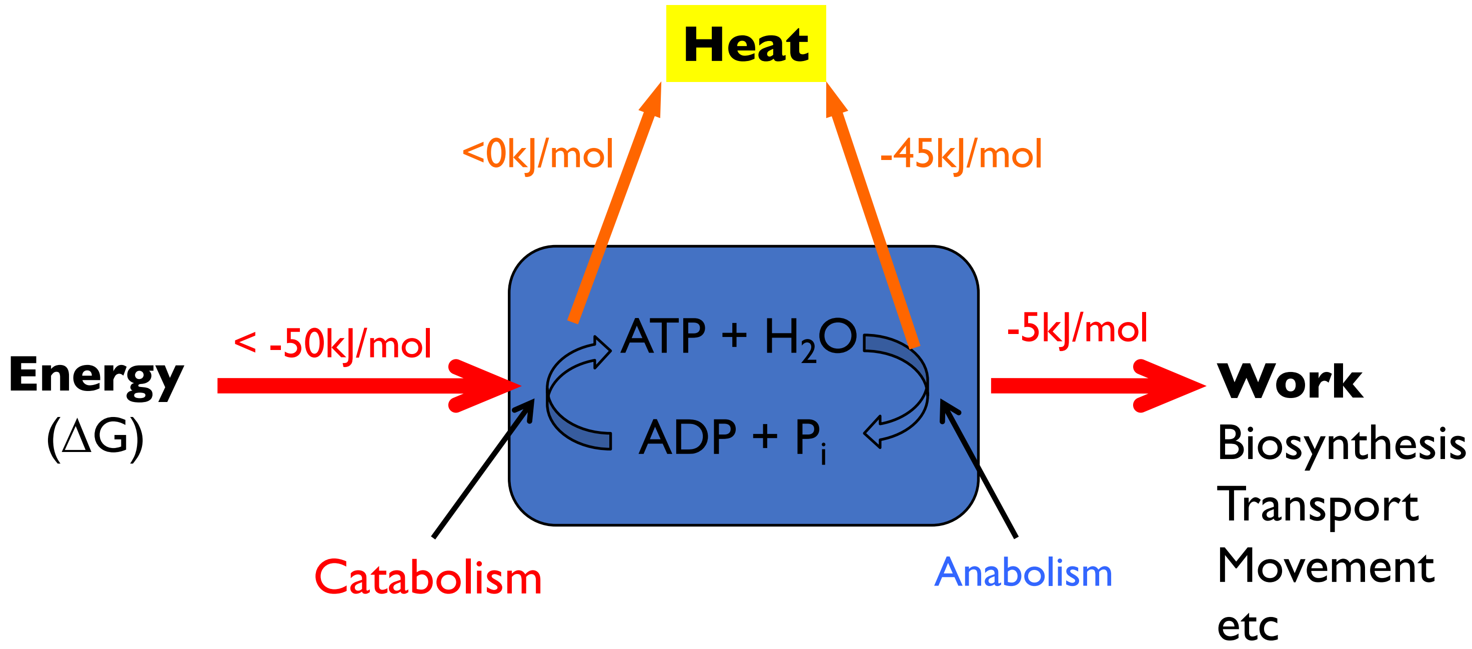
Energy source	Catabolic Electron Donor/Acceptor	Carbon source	Microorganism
Photo-	Litho (S_1 AND S_2)	auto-	
			troph
Chemo-	organo (S_1 OR S_2)	hetero-	

S_1	**S_2**	**Catabolism**
organic	Organic/inorganic	chemoorganotroph
inorganic	organic	chemoorganotroph
inorganic	inorganic	chemolithotroph
	$=O_2$	aerobic
	$\neq O_2$	anaerobic
	$=O_2$ or $\neq O_2$	facultative

Carbon source	**Anabolism**
> 50% CO_2	autotroph
organic (<>0%)	heterotroph

How Microbes use Energy

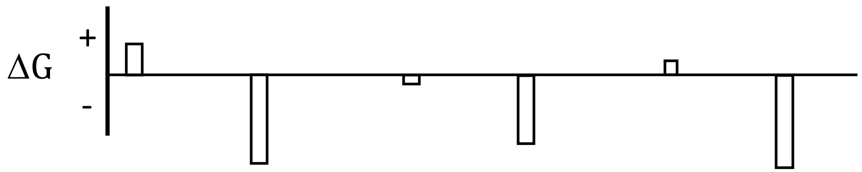
Use of Gibbs Free Energy as a Resources



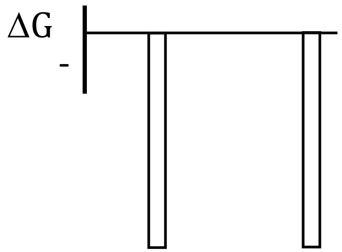
A $S \longrightarrow P$ $\Delta G_{S\text{->}P} < 0$

B $S \longrightarrow I_1 \longrightarrow I_2 \longrightarrow I_3 \longrightarrow I_4 \longrightarrow I_5 \longrightarrow P$

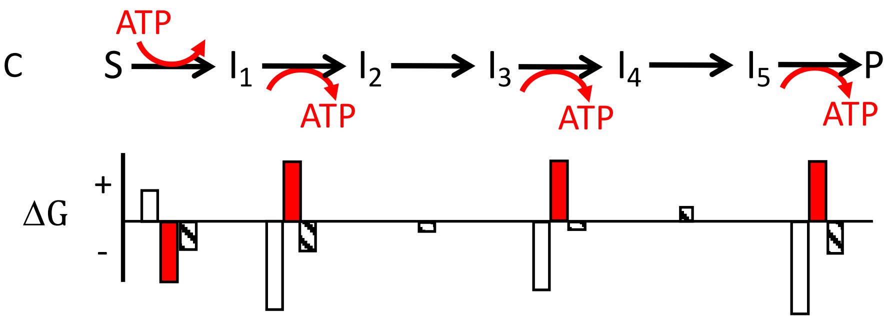
$\Delta G_{S\rightarrow I_1}$ $\Delta G_{I_1\rightarrow I_2}$ $\Delta G_{I_2\rightarrow I_3}$ $\Delta G_{I_3\rightarrow I_4}$ $\Delta G_{I_4\rightarrow I_5}$ $\Delta G_{I_5\rightarrow P}$



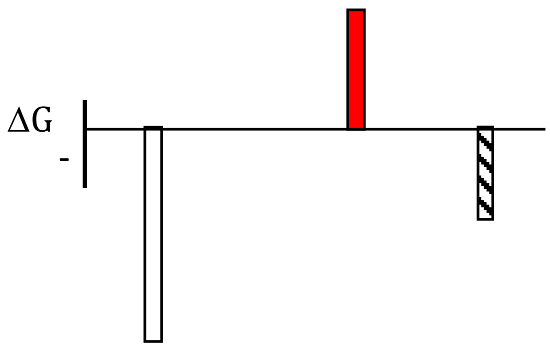
$\Delta G_{S\rightarrow P} = \Sigma \Delta G_{I_{1-6}}$



C



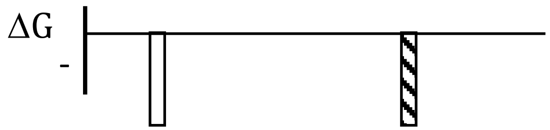
$$\Delta G_{S\text{->}P} = n_{ATP}\Delta G_{ATP} + \Delta G_{heat}$$



D

$$S + nADP + nP_i \longrightarrow P + nATP$$

$$\Delta G_{S\text{->}P,\ coupled} = \Delta G_{heat}$$



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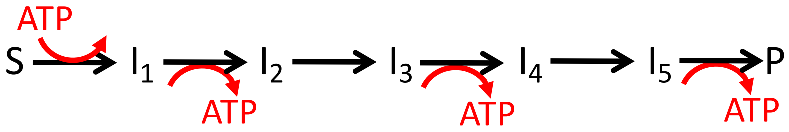
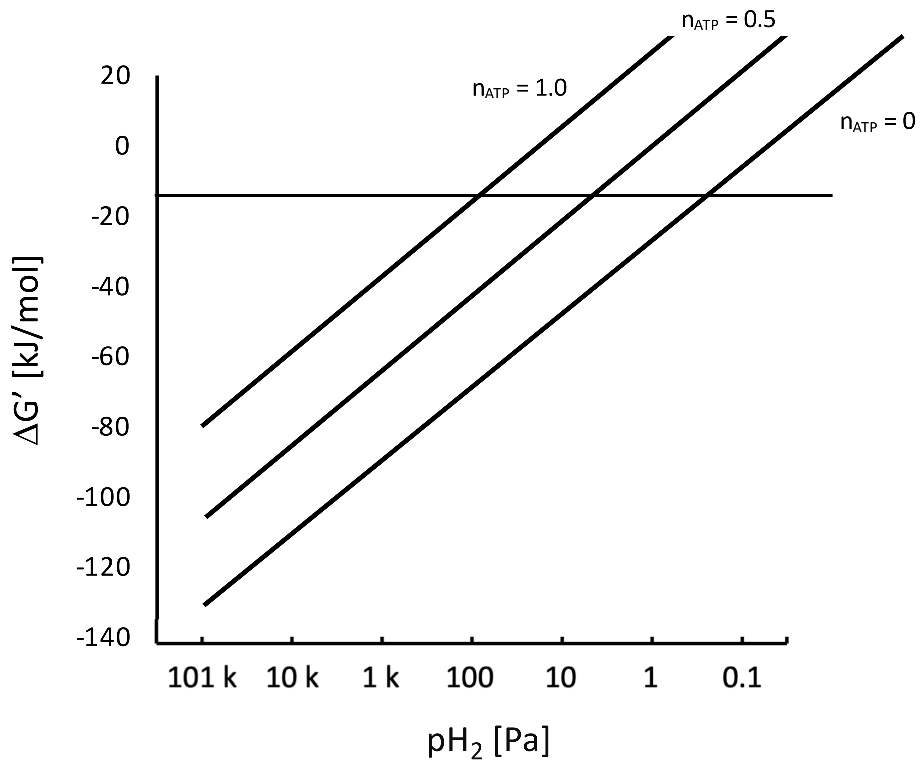


$$\Delta G_{S\text{->}P} = n_{ATP}\Delta G_{ATP} + \Delta G_{heat}$$

$$4\ H_2 + CO_2 + n_{ATP}\ ADP + n_{ATP}\ P_i \rightarrow CH_4 + n_{ATP}\ ATP + 3\ H_2O$$

$\Delta G'_{nATP=0}$ - 131 kJ/mol



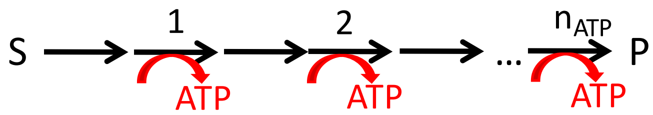
A link between thermodynamics (n_{ATP}) and kinetics (flux J_S)

Ecological success and microbial growth

- Relationship between growth, available energy, and growth rate -

Available energy: $$\Delta G_{net} = \Delta G_{S \to P} - n_{ATP} \Delta G_{ATP}$$

In thermodynamic equilibrium, $\Delta G_{net} = 0$, and $n_{\max} = \frac{\Delta G_{S \to P}}{\Delta G_{ATP}}$



Pfeiffer & Bonhoeffer 2002,
Costa et al 2006

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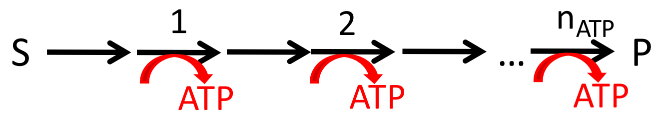


Fluxes (rates):

$$J_S = \frac{dS}{dt} \qquad J_{ATP} = n_{ATP} J_S$$

In thermodynamic equilibrium, J_S and $J_{ATP} = 0$

However at yields n_{ATP} lower than n_{max}, ΔG_{net} = <0 and drives the reaction, and consequently, J_S and J_{ATP} become positive.

$$J_S \propto \Delta G_{net}$$

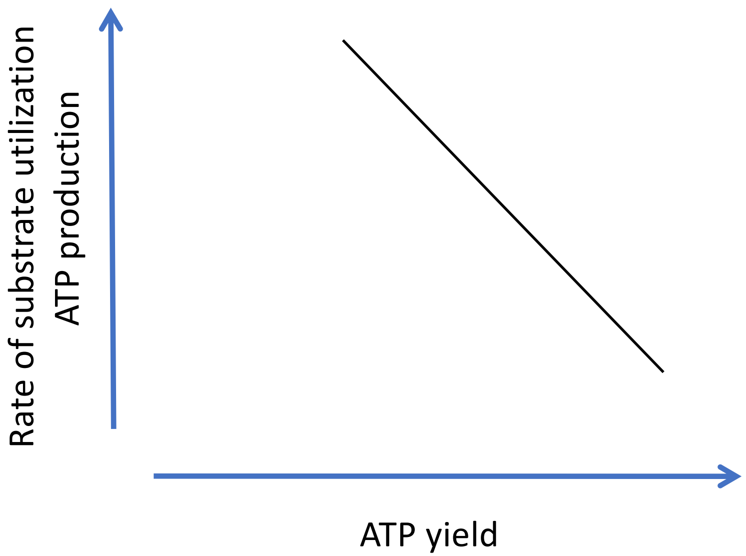
$$J_S = k_1 (n_{\max} - n_{ATP}) \qquad J_{ATP} = k_1 n_{ATP} (n_{\max} - n_{ATP})$$

Pfeiffer & Bonhoeffer 2002,
Costa et al 2006

Rate *versus* yield tradeoff

- for fundamental thermodynamic reasons, irrespective of the pathway -

$$J_S = k_1\left(n_{\max} - n_{ATP}\right) \qquad J_{ATP} = k_1 n_{ATP}\left(n_{\max} - n_{ATP}\right)$$



Pfeiffer & Bonhoeffer 2002

Competition favors rate over yield

P = pay-off, total amount of ATP produced from a given amount of resource S for a given strategy n_{ATP}

$$P(n_{ATP}) = tJ_{ATP}(n_{ATP}) \qquad S = tJ_S(n_{ATP})$$

P under non-competitive conditions

$$P(n_{ATP}) = S\frac{J_{ATP}(n_{ATP})}{J_S(n_{ATP})} = S \bullet n_{ATP}$$

Pay-off for a given strategy is determined only by its yield but not its rate of ATP production

P under competitive conditions

$$P(n_{ATP}) = S\frac{J_{ATP}(n_{ATP})}{J_S(n_{ATP}) + J_c} \qquad P(n_{ATP}) = S\frac{k_1 n_{ATP}(n_{\max} - n_{ATP})}{k_1(n_{\max} - n_{ATP}) + J_c}$$

If J_C is small, P is large if J_S is small; i.e., if competition is small, a slow strategy for efficient ATP formation is advantageous.

If J_C is large, a more efficient pathway (n_{ATP}) will not significantly reduce J_S, and strategies with high rates but lower yields will result in higher pay-off.

Size of a population is determined by yield;
outcome of competition is determined by rate of ATP production

Pfeiffer & Bonhoeffer 2002,

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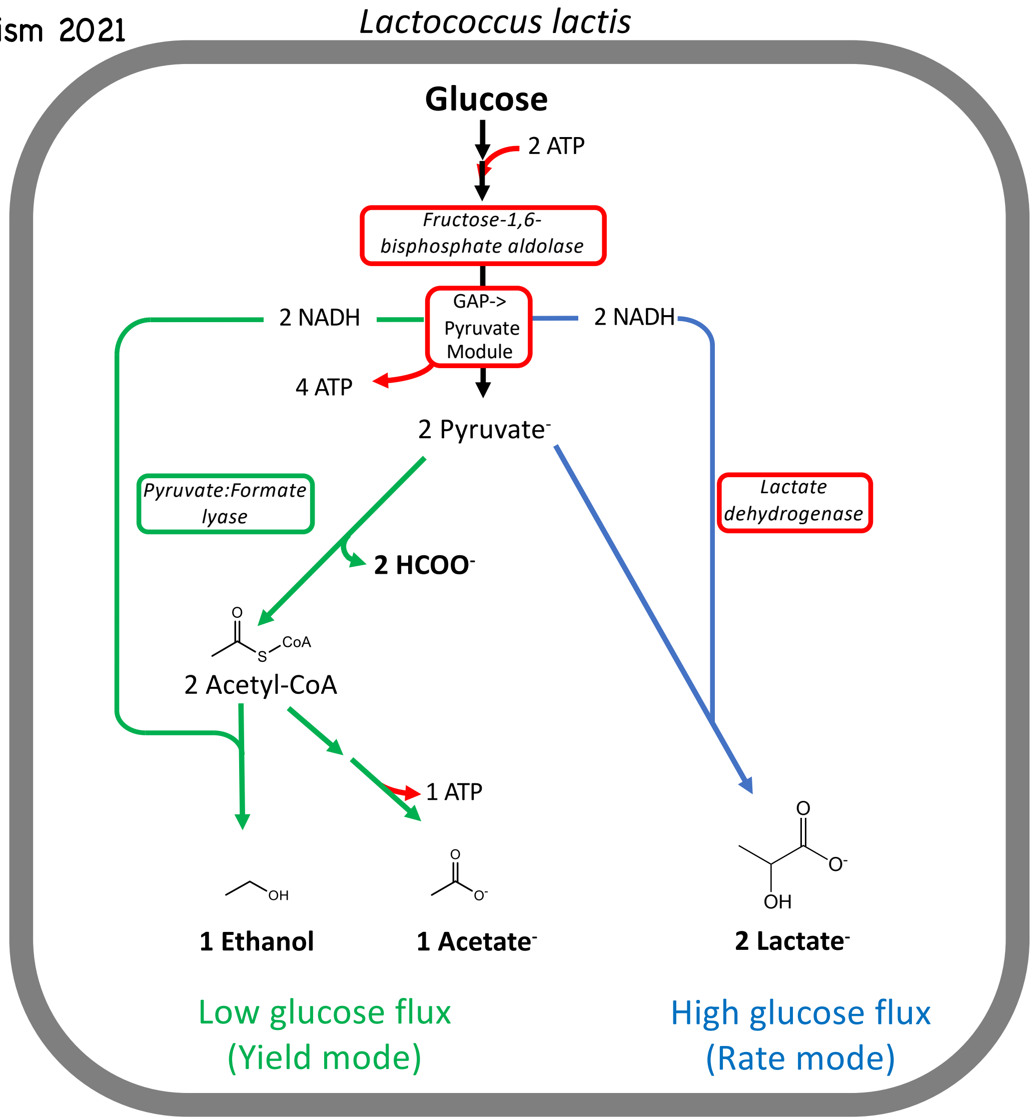

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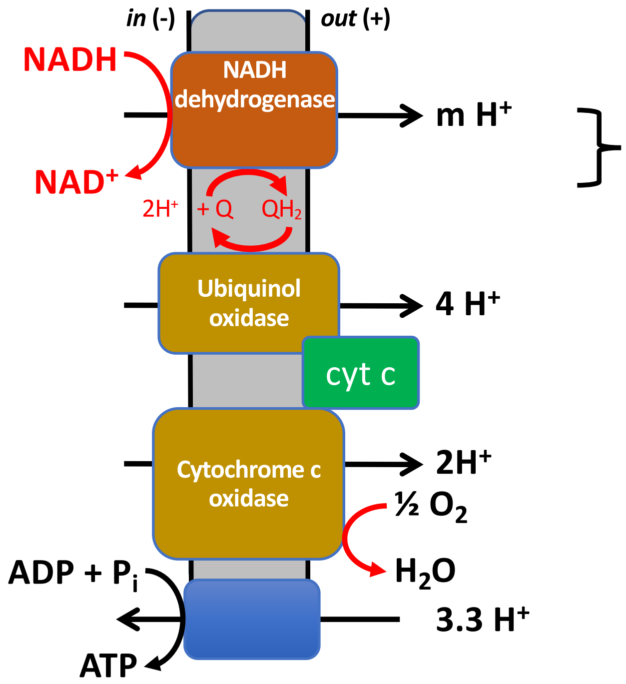
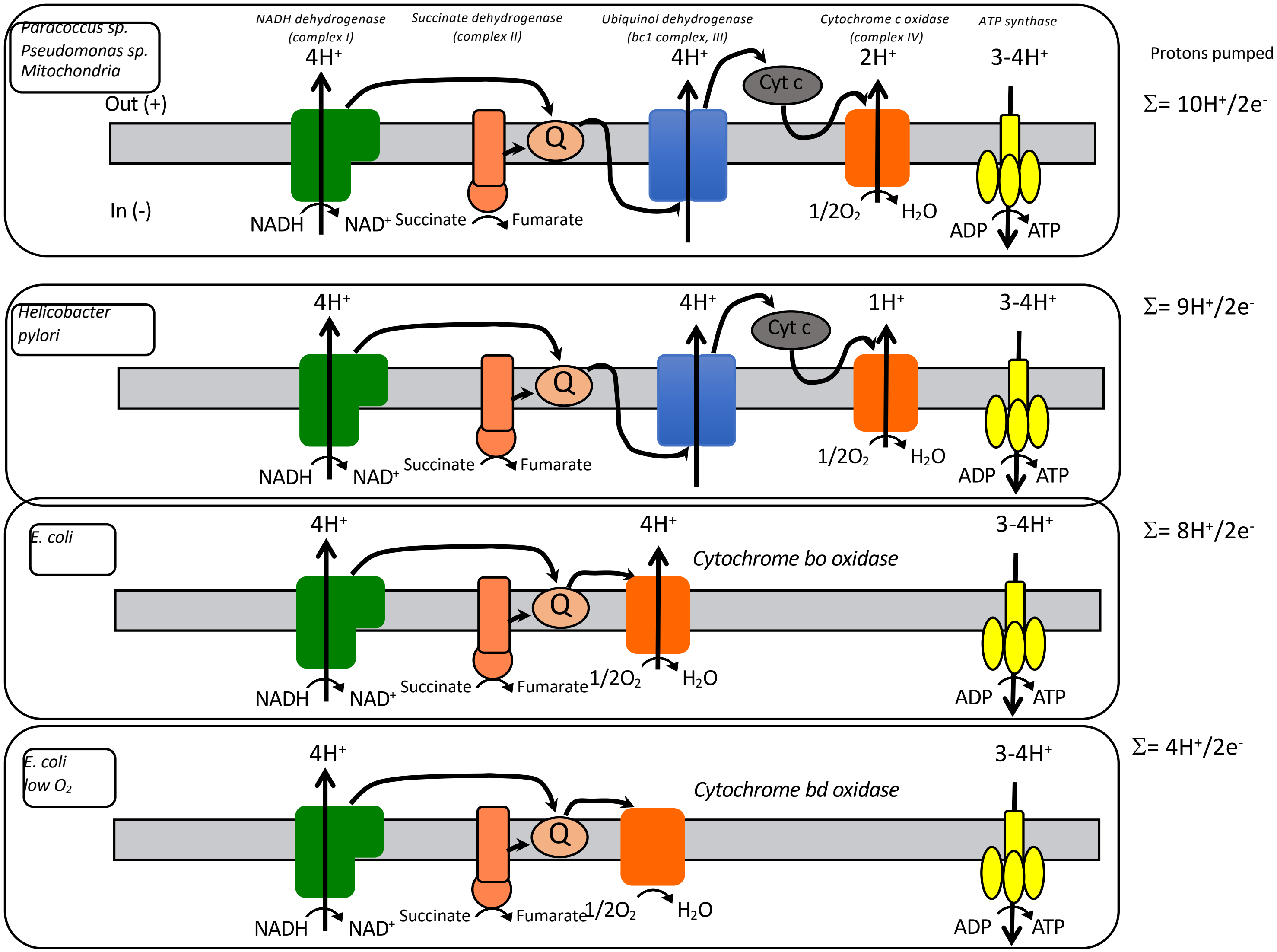
NADH + ubiquinone → NAD^+ + Ubiquinol $\Delta G^{0'}$ = - 61kJ/mol NADH



NDH 1	m = 3-4 H^+
NDH 2	m = 0 H^+
Nqr	m = 2 Na^+

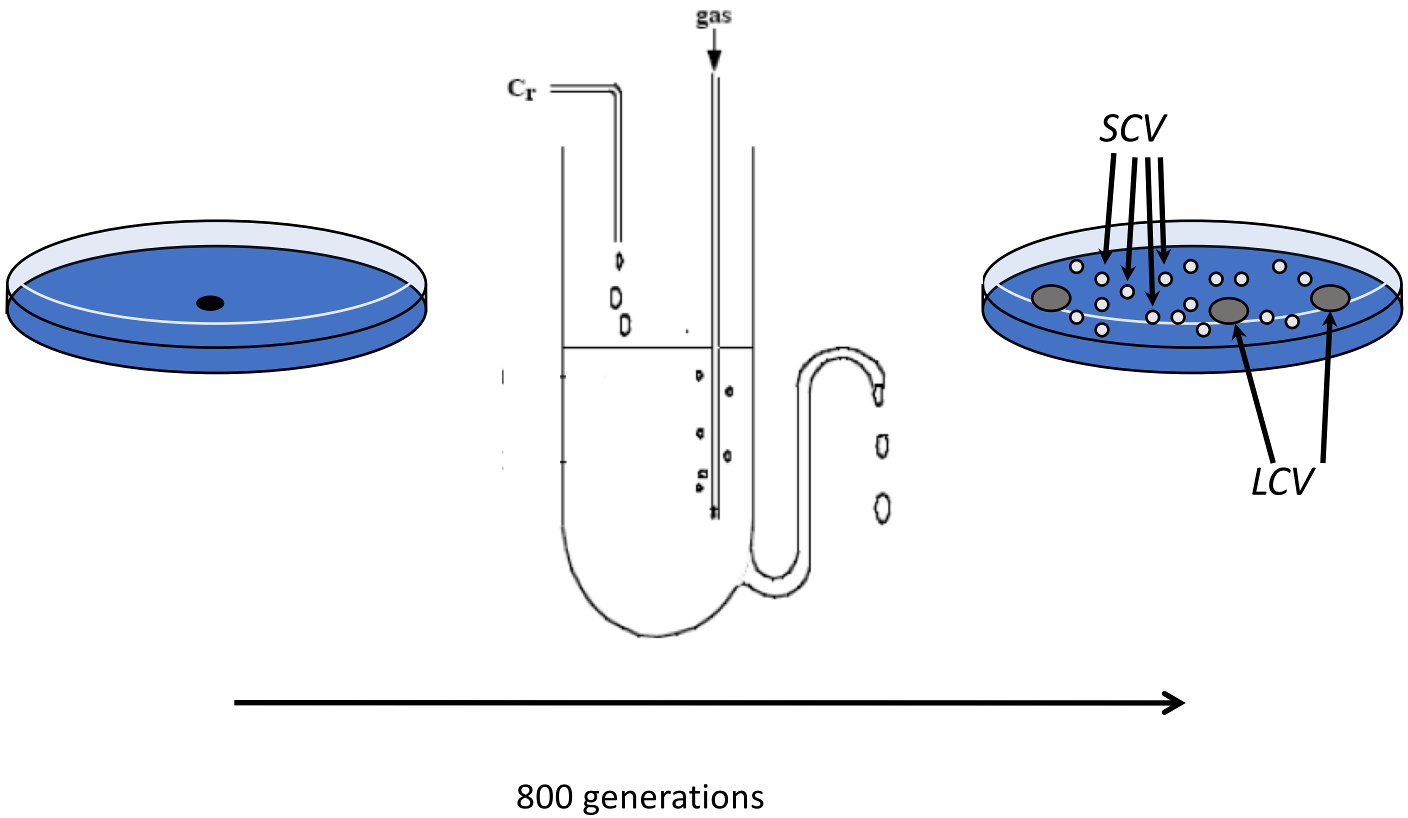


Paracoccus sp.
Pseudomonas sp.
Mitochondria
NADH dehydrogenase (complex I)
Succinate dehydrogenase (complex II)
Ubiquinol dehydrogenase (bc1 complex, III)
Cytochrome c oxidase (complex IV)
ATP synthase
Protons pumped
Out (+)
In (-)
4H+
4H+
2H+
3-4H+
Cyt c
Q
NADH
NAD+
Succinate
Fumarate
1/2O2
H2O
ADP
ATP
Σ= 10H+/2e-
Helicobacter pylori
4H+
4H+
1H+
3-4H+
Σ= 9H+/2e-
E. coli
4H+
4H+
Cytochrome bo oxidase
3-4H+
Σ= 8H+/2e-
E. coli
low O2
4H+
Cytochrome bd oxidase
3-4H+
Σ= 4H+/2e-

Speciation of Pathways
(i. e., metabolic interactions)

Metabolism and Ecological Patterns

Julian Adams experiment: *E. coli* in a glucose-limited chemostat (simplified)

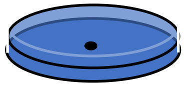


Helling, Vargas, Adams 1987

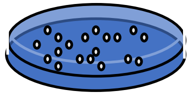
Julian Adams experiment: *E. coli* in a glucose-limited chemostat

Growth characteristics

Ancestral strain

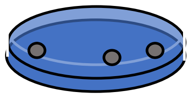


Small Colony Variant

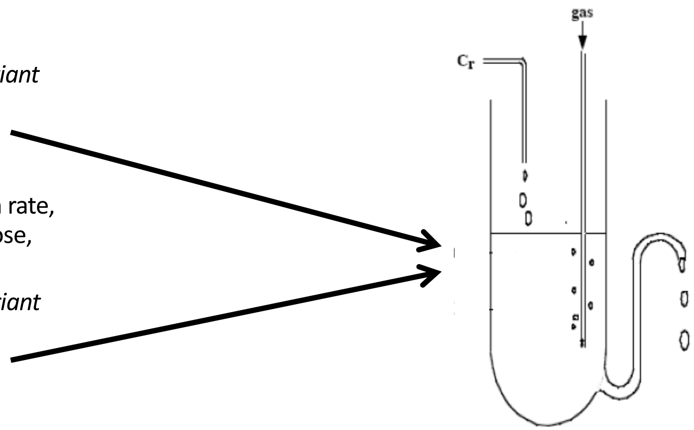


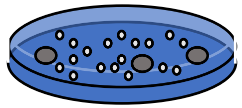
Higher max growth rate,
Lower Km for glucose,

Large Colony Variant



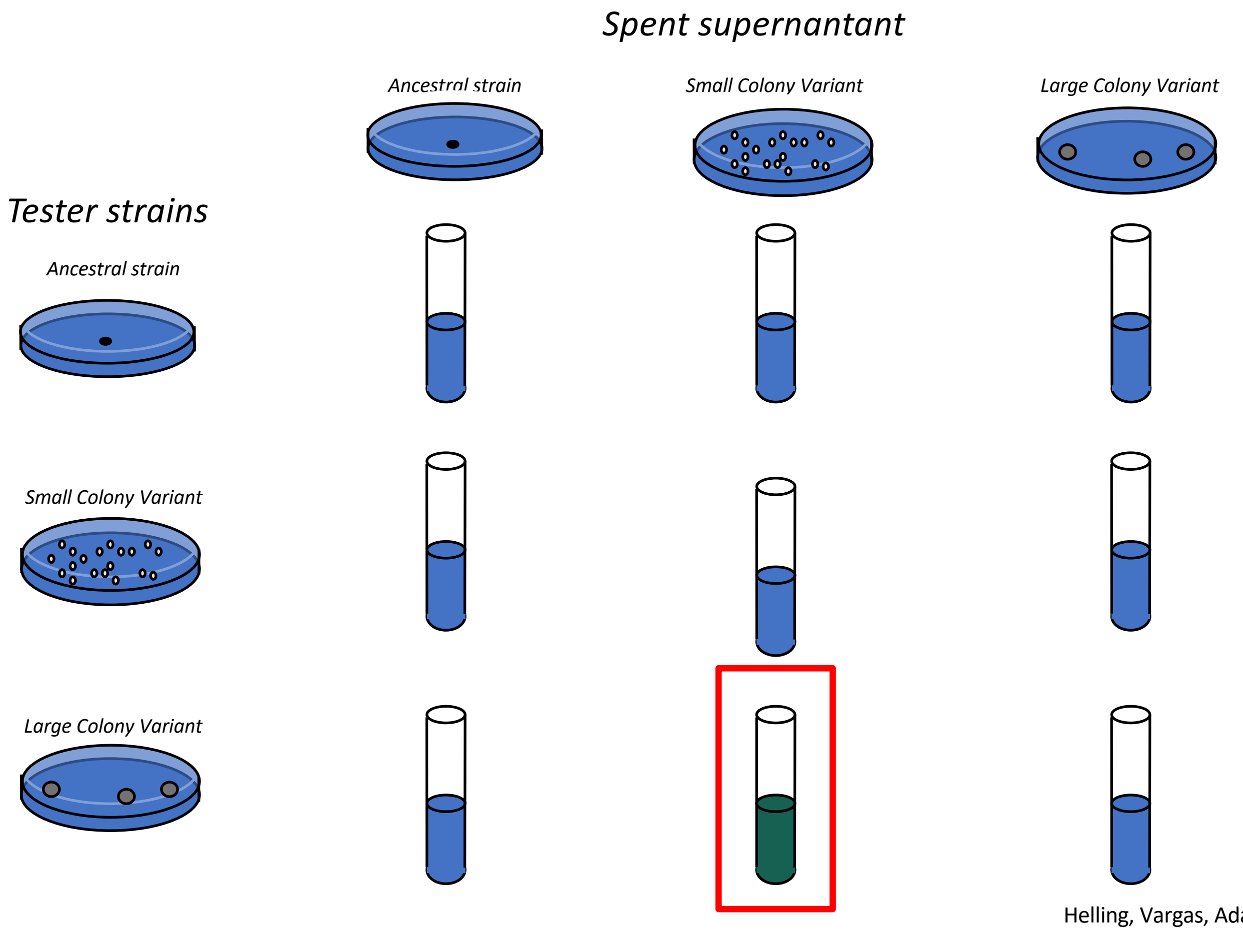
Higher relative growth yield





Both populations are stably maintained

Growth on spent media supernatant: Metabolic cross-feeding, niche creation, and speciation in the evolved E. *coli* variants.



Helling, Vargas, Adams 1987

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What the outcome of the Julian Adams experiment means:

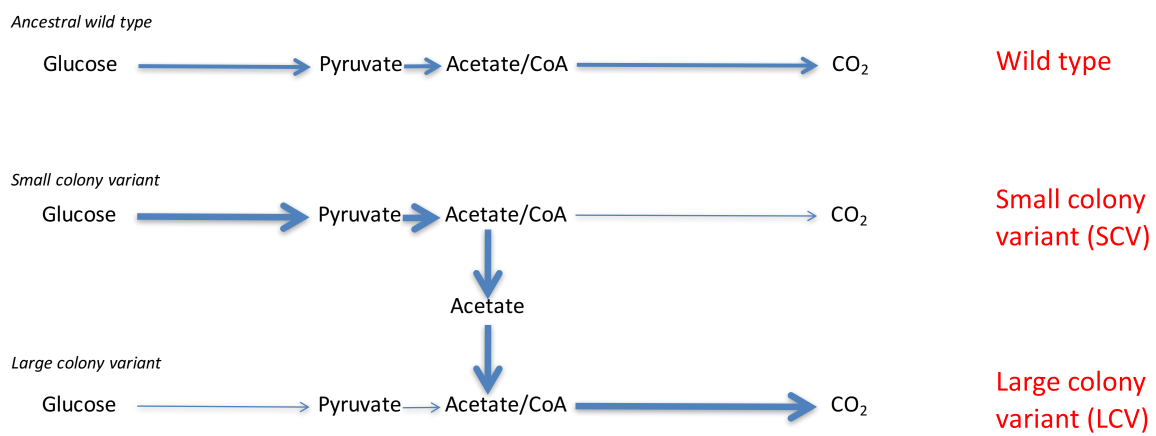
•Small colony variants appear to be a predictable feature of adaptation to a glucose-limited environment.

•Stable (predictable) environments lead to speciation.

•SCV has superior glucose uptake but also utilizes glucose inefficiently.

•Some evolved organisms are more successful *because* they use the substrate less well.

•The less efficient types are maintained because they do not compete for glucose but rather grow on the excreted metabolite.



Real World Examples of Pathway Speciation

Anoxic organic-rich environments:

Low flux, low competition: **Glucose** $\xrightarrow{\text{Propionic acid bacterium}}$ **Propionate⁻, Acetate⁻**

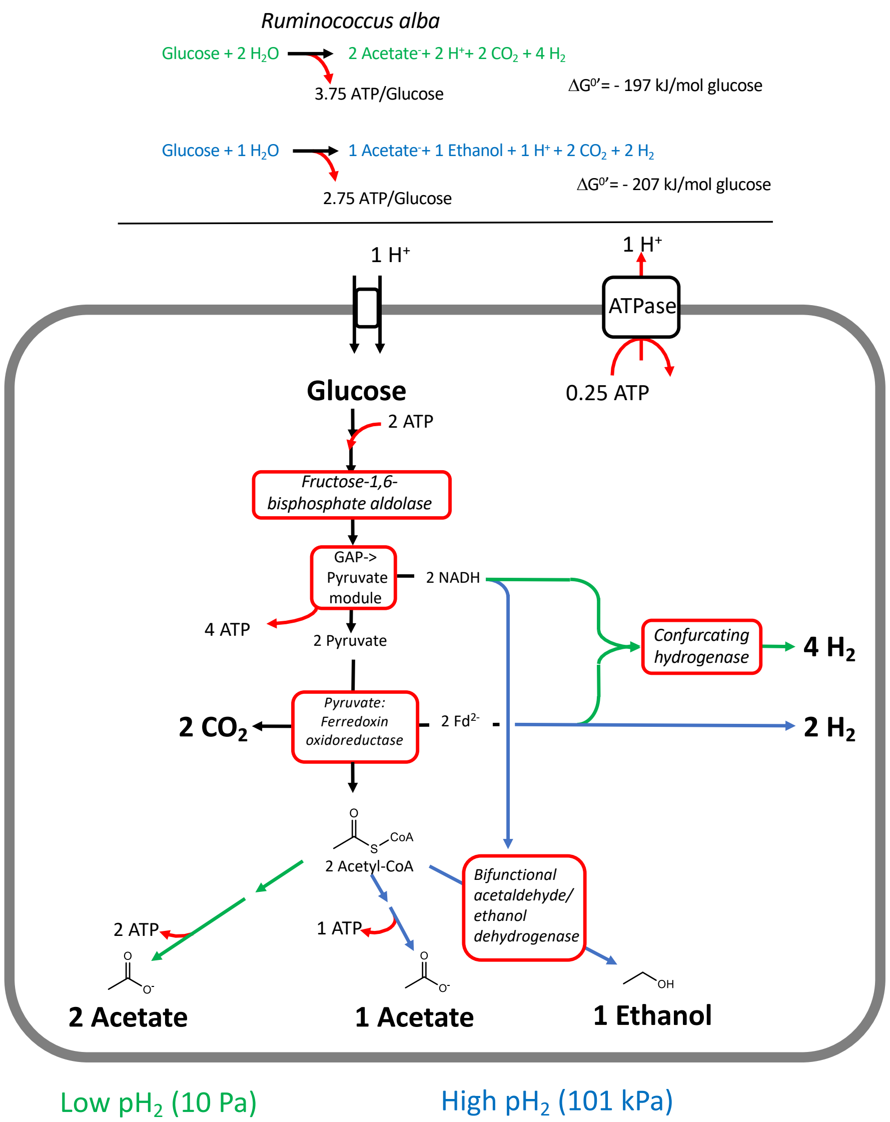
High flux, high competition: **Glucose** $\xrightarrow[\text{bacterium}]{\text{Lactic acid}}$ **Lactate⁻** $\xrightarrow[\text{bacterium}]{\text{Propionic acid}}$ **Propionate⁻, Acetate⁻**

Glucose $\xrightarrow{\textit{S. gordonii}}$ 2 Lactate⁻ + 2 H^+

2 Lactate⁻ $\xrightarrow{\textit{V. atypica}}$ 2 Propionate⁻ + 1 Acetate⁻ + CO_2

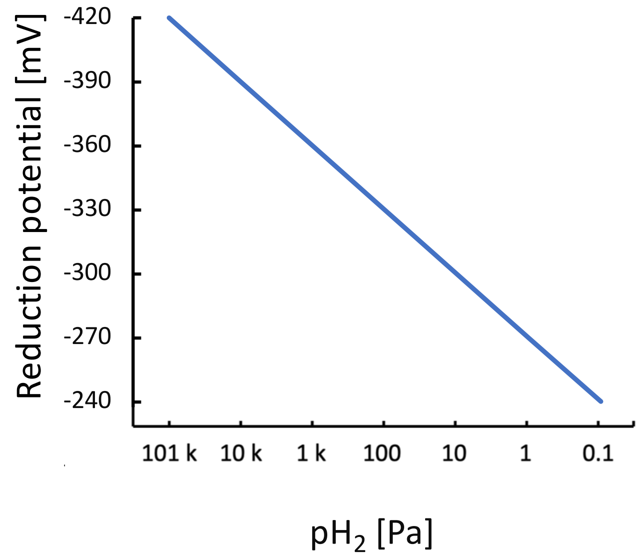
+ *amyB*

Glucose $\xrightarrow{\textit{S. gordonii}}$ 2 Lactate⁻ $\xrightarrow{\textit{V. atypica}}$ 2 Propionate⁻ + 1 Acetate⁻ + CO_2



H_2 Partial Pressure (pH_2) and the Redoxpotential of H_2

$$2\,e^- + 2\,H^+ \rightleftharpoons H_2$$



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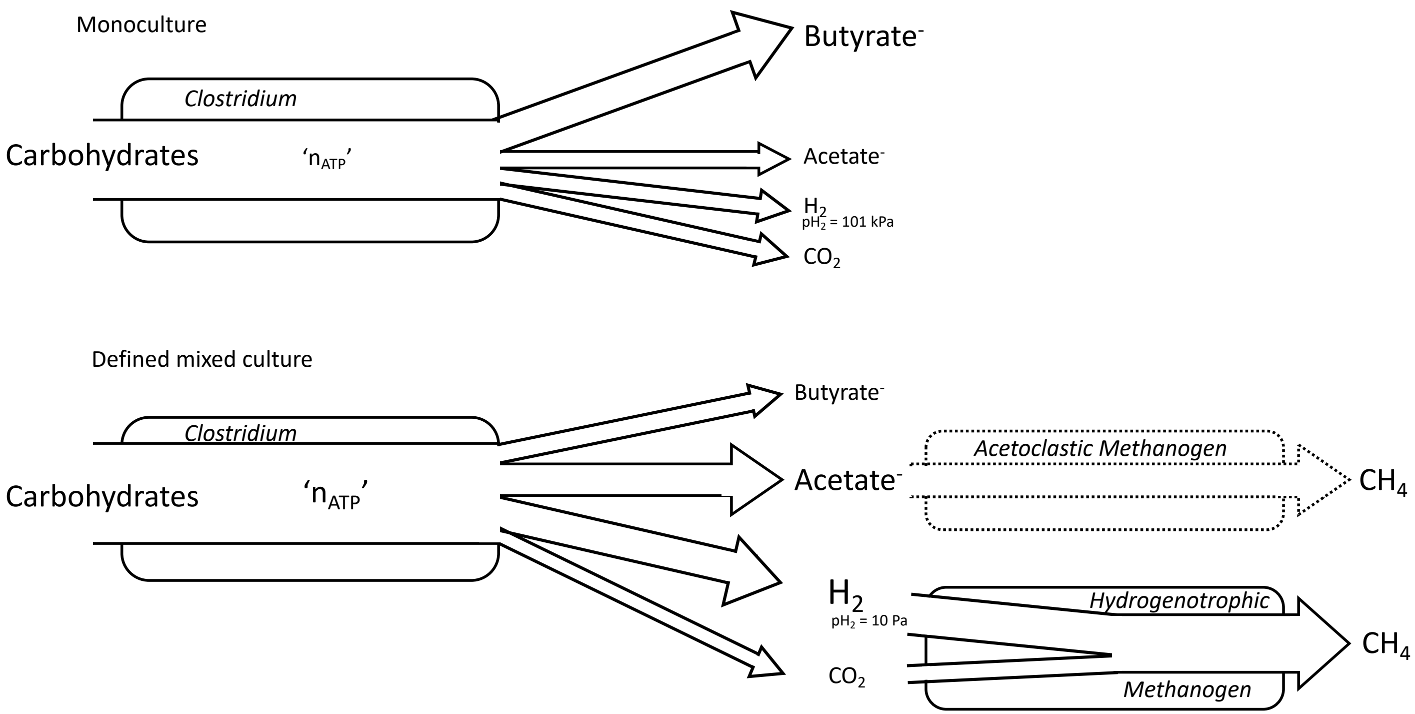

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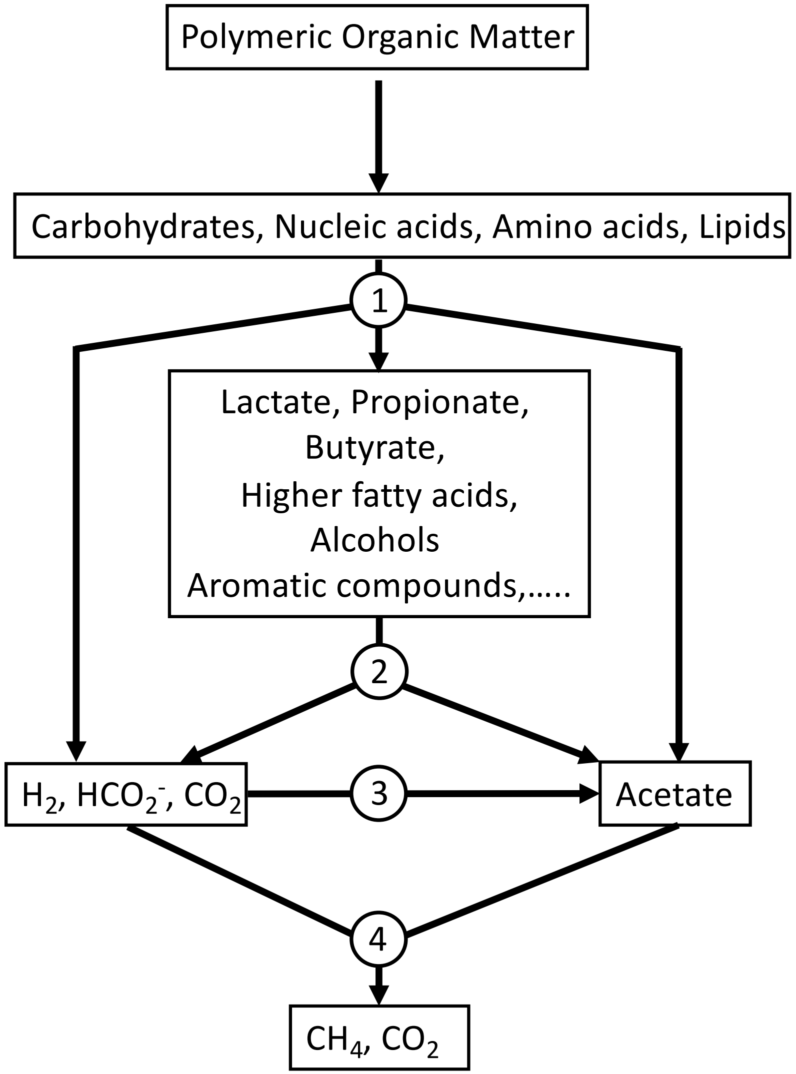
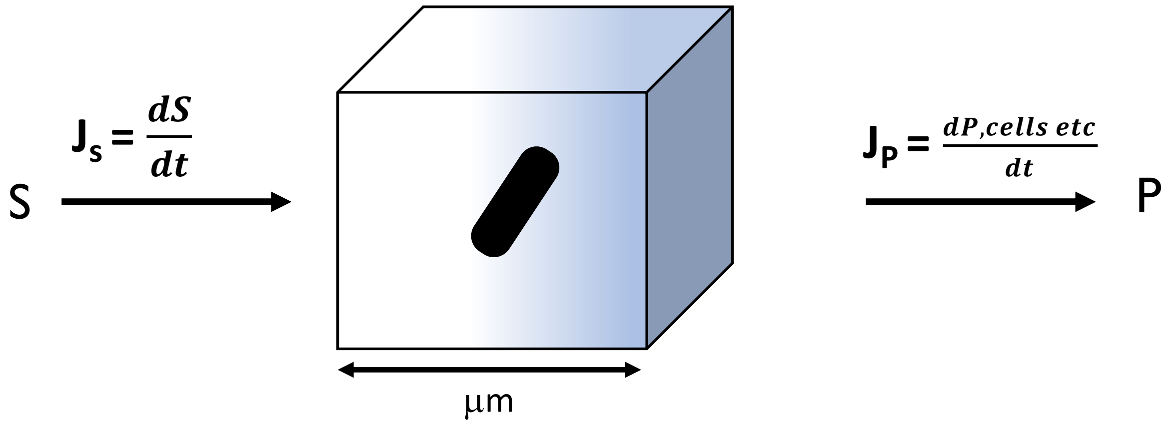
Flow of Electrons and Carbon under Methanogenic Conditions



Selection, Isolations, and Metabolism

A microbe's environment



A Microbe's Environment consists of:
- A spatial (μm) component
- At least one catabolic resource
- A flux of resources

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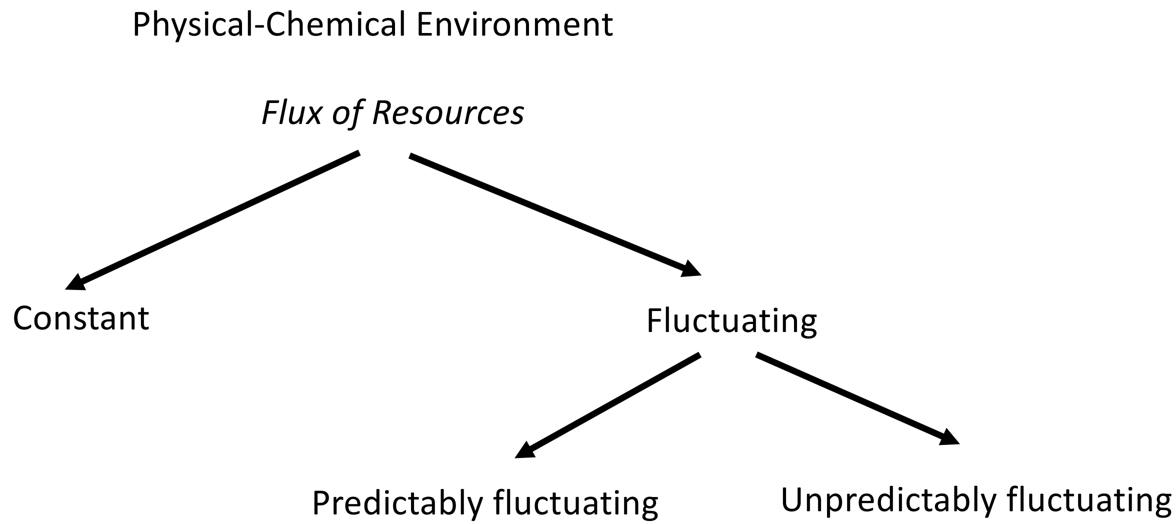

Selection

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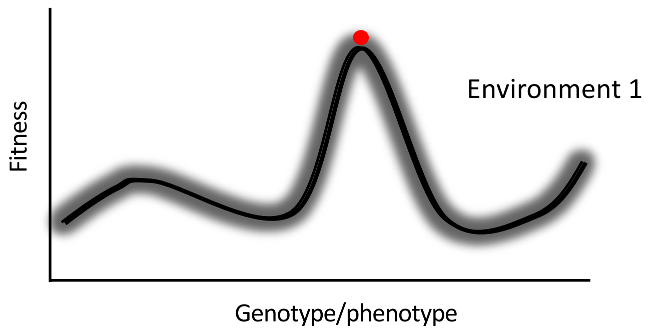
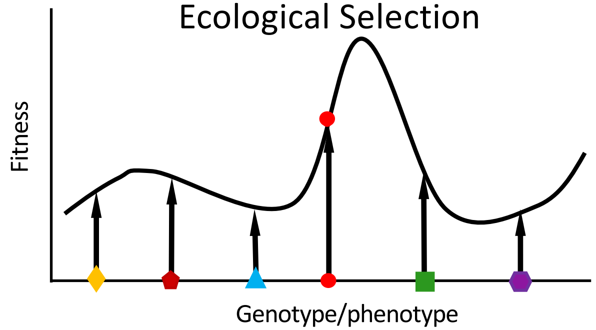
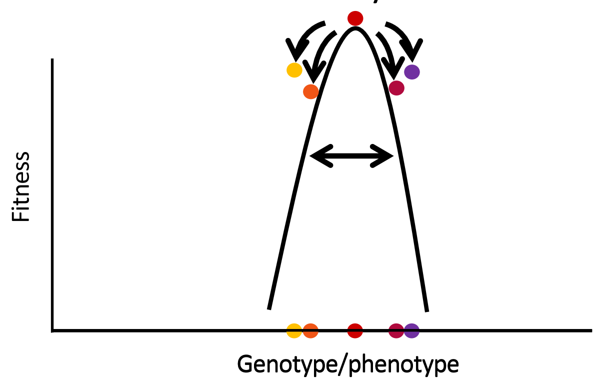
Fitness landscape



Ecological Selection

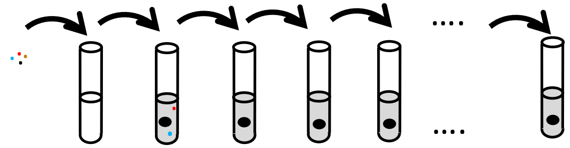


Evolutionary Selection



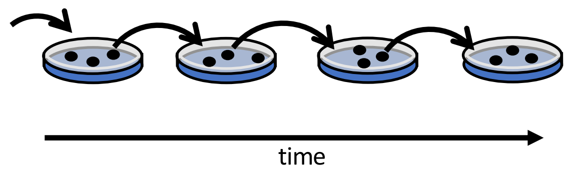
Isolation of Microbes and Selection

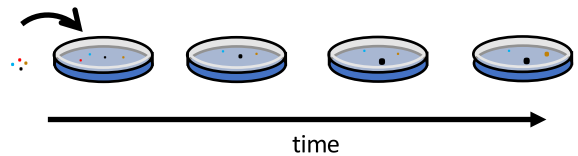
Liquid enrichments



Competition is global;
Selection under competition favors fast growing microbes (high $J_{S,}$ high J_{ATP})

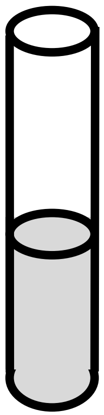
Direct isolations

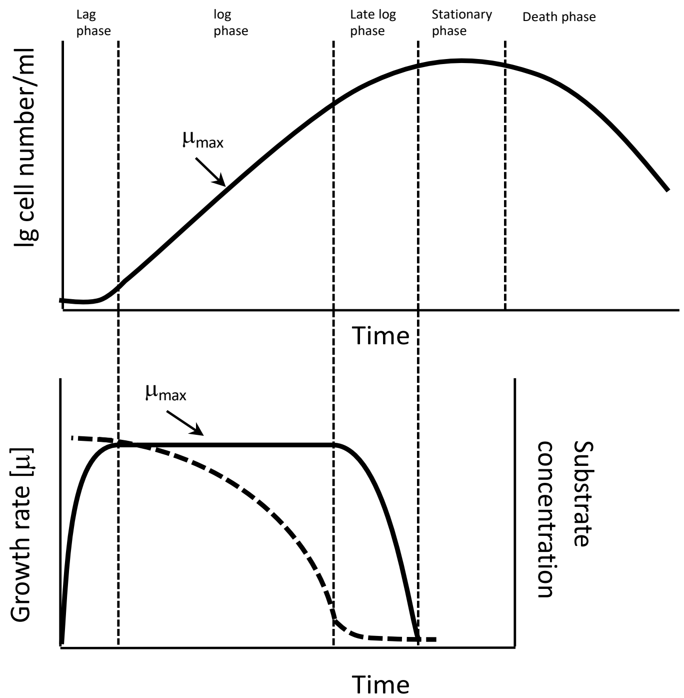




Competition is local (initially)
Effect of Drift

Growth in Batch





Growth in batch selects for the fastest microbes (dATP/dt)

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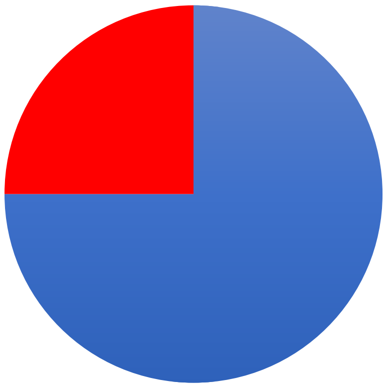
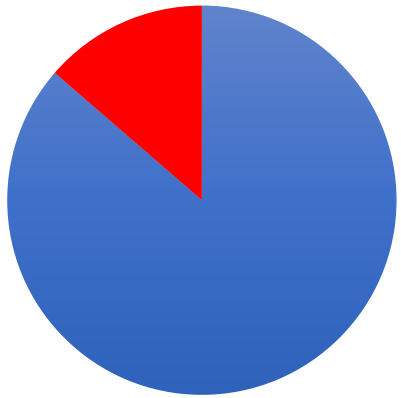
Proteome allocation

E. coli: Glucose + 6 O_2 → 6 CO_2 + 6 H_2O $\Delta G^{0\prime}$ ~ 2800 kJ/mol

Slow growing cell $J_{ATP\ s}$



Fast growing cell $J_{ATP\ f}$



Catabolic

The proteome dedicated to energy conservation for the anabolic demand, i.e., growth rate

Anabolic (including ribosomes)

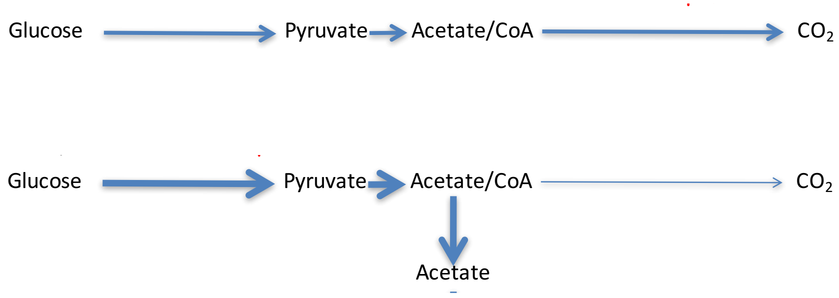
The proteome dedicated to biosynthesis is linearly proportional to growth rate.

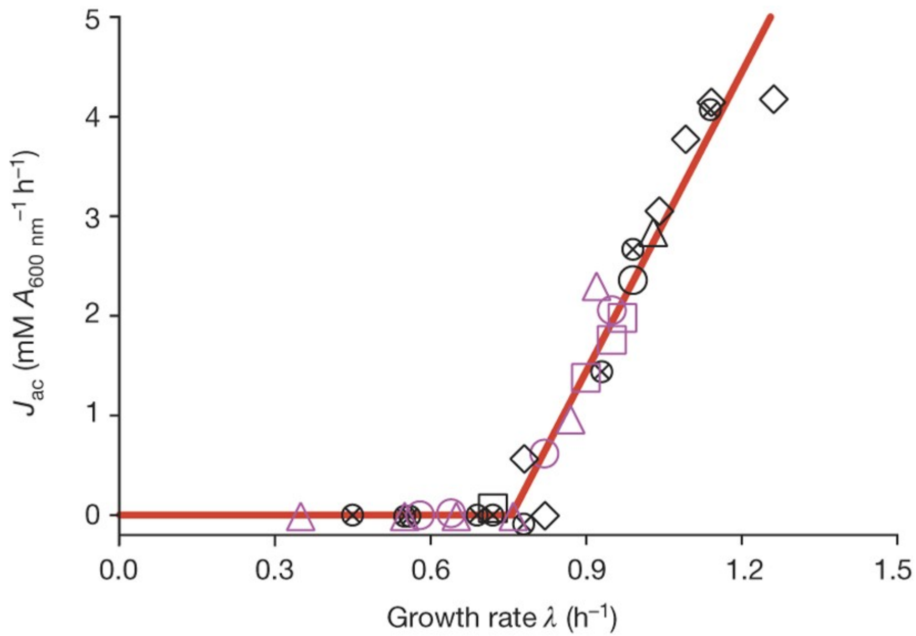
$J_{ATP\ f} > J_{ATP\ s}$

A smaller catabolic proteome needs to mediate a higher ATP flux

Terry Hwa & coworkers

Acetate Overflow Metabolism in *E. coli*





Basan et al 2015

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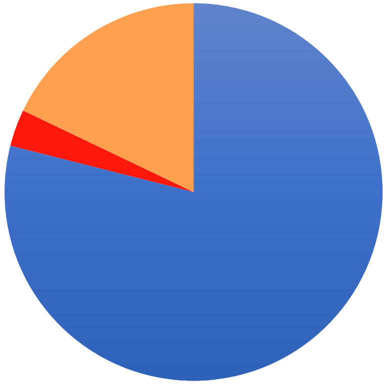
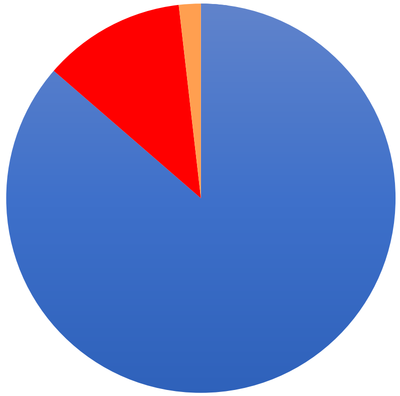
Proteome allocation

Slow growing $J_{ATP\,s}$



Fast growing $J_{ATP\,f}$



Respiration

Fermentation

Anabolic (including ribosomes)

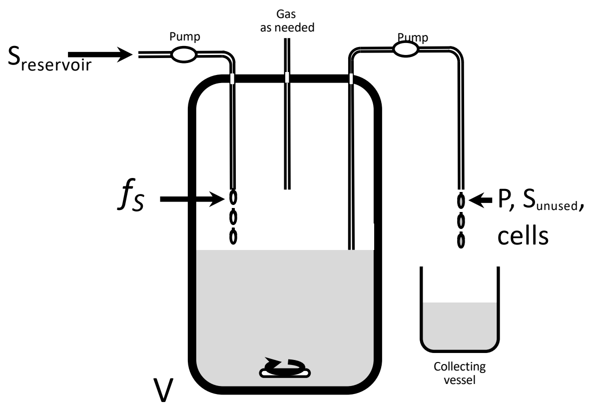
The proteome dedicated to biosynthesis is linearly proportional to growth rate.

Catabolic

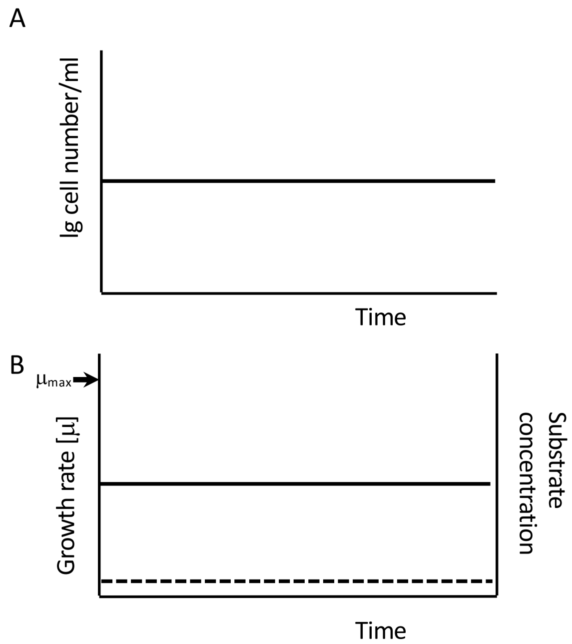
The proteome dedicated to energy conservation for the anabolic demand, i.e., growth rate

Terry Hwa & coworkers

Growth in Chemostat



$$D = \frac{f_S}{V} = \mu$$



Growth in chemostat selects for the 'efficient' microbes