Chemostat Models of Yeast Growth: Respiratory Oscillation *vs.* Aerobic Glycolysis

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Contents

1 Introduction

Here, we explore the simplest possible extensions to the classical chemostat model that are required to describe observations of yeast growth: aerobic glycolysis at high dilution rates and purely respiratory or oscillating states at low dilution rates.

The fundamental overall feedbacks of cellular growth and metabolism are the focus, and only very coarse representations of intracellular dynamics are used.

A top-down approach is followed. We start from Monod's basic growth model, analyze the feedback structure and regulatory potential present at a given level, and then extend the model to accomodate deviating or more detailed observations.

The base models should all be analyzed separately, but can be combined. Full explicit models are described in the Appendix.

Physiological "why?" questions are "what and how?" questions on evolutionary timescale: optimization (ressource allocation, trade-off) questions can be asked at each level; by simple numerical chemostat competition or evolution experiments, and where possible analytical solution of the equations.

And finally, once dynamics on reactor-level are understood, a more detailed model of intracellular dynamics can be used to study effects of storage or overflow metabolism. Bottom-up.

Ideally, the approaches are coupled formally, e.g., by using FBA from a detailed stoichiometric metabolic model to generate the biomass functions of the coarse-grained dynamic model. In turn, the empirial dependencies of reactor-level rates shall be translated to kinetics and mechanistics (regulatory interactions) of the detailed flux model.

1.1 General Ideas

The general motivation is to find a reasonable model that can account for:

1. synchronized oscillations at low dilution rates ($< D_{crit}$) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10] with period dependencies on dilution and aeration rates, and nutrient or product concentrations,

2. aerobic glycolysis at high dilution/growth rates and high glucose concentrations ($> D_{crit}$, Crabtree effect) [11, 12, 13],

3. multistability and low relaxation times around D_{crit} [11, 12, 14],

4. increasing fraction of aerobic glycolysis at constant growth rate during decreasing glucose concentration in batch growth [15], and

5. both, oscillation in diverse conditions and the linear relations of cluster A/AB (pos.) and D (neg.) gene expression with growth rate.

Oscillatory Growth. At low dilution rates glucose-limited steady state cultures are observed to grow exclusively respiratory [1, 11, 16, 17, 18, 19, 20, 12, 21, 13] and the respiratory quotient of the culture is RQ=1. However, during these conditions and especially when the reactor is well-mixed, the cells tend to partially synchronize their cell division cycles [2, 19]. In self-synchronized growth cells ferment and produce ethanol during the budding phase. This ethanol is thought to be consumed immediately by unbudded cells [19], but there is net ethanol accumulation and the average RQ>1. Thus, oscillatory cultures are fermenting part of the glucose even at low growth rates.

However, several observations question the model of cell cycle self-synchronization:

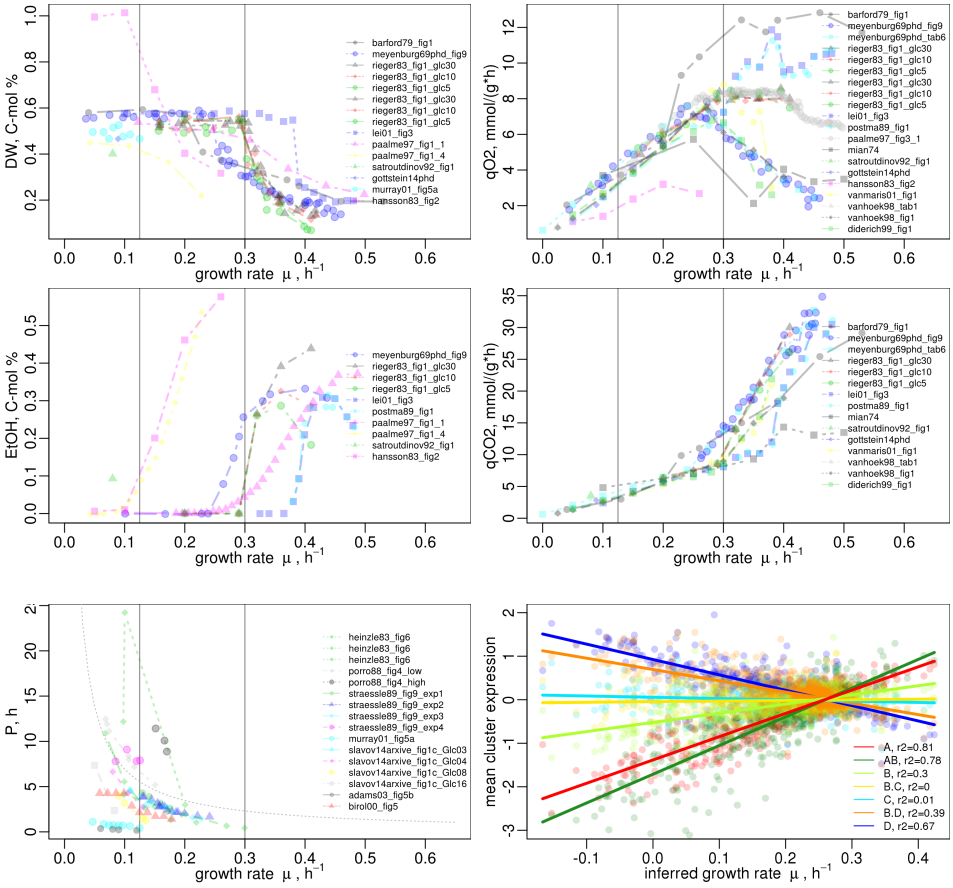
1. oscillatory growth was also observed when cells are grown on ethanol, where unlike oscillations on glucose, no accumulation of storage carbohydrates was observed [22],

2. oscillations are also observed for non-growing (but perhaps very slowly growing?) cells [23],

3. de-synchronized oscillations are observed in single cells in steady-state cultures [24],

4. the strain IFO 0233 shows oscillations at periods that are shorter then any so far observed budding times [3] and only a tenth of the culture doubling time.

These discrepancies gave rise to the idea that there is an autonomic metabolic or respiratory oscillation that gates the cell cycle or S-phase, and is based on intracellular regulation. Can we reconcile these observations?


DW, C-mol %
growth rate μ , h^{-1}
barford79_fig1
meyenburg69phd_fig9
rieger83_fig1_glc30
rieger83_fig1_glc10
rieger83_fig1_glc5
rieger83_fig1_glc30
rieger83_fig1_glc10
rieger83_fig1_glc5
lei01_fig3
paalme97_fig1_1
paalme97_fig1_4
satroutdinov92_fig1
gottstein14phd
murray01_fig5a
hansson83_fig2
qO2, mmol/(g*h)
growth rate μ , h^{-1}
barford79_fig1
meyenburg69phd_fig9
meyenburg69phd_tab6
rieger83_fig1_glc30
rieger83_fig1_glc10
rieger83_fig1_glc5
rieger83_fig1_glc30
rieger83_fig1_glc10
rieger83_fig1_glc5
lei01_fig3
postma89_fig1
paalme97_fig3_1
mian74
satroutdinov92_fig1
gottstein14phd
hansson83_fig2
vanmaris01_fig1
vanhoek98_tab1
vanhoek98_fig1
diderich99_fig1
EtOH, C-mol %
growth rate μ , h^{-1}
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rieger83_fig1_glc30
rieger83_fig1_glc10
rieger83_fig1_glc5
lei01_fig3
postma89_fig1
paalme97_fig1_1
paalme97_fig1_4
satroutdinov92_fig1
hansson83_fig2
qCO2, mmol/(g*h)
growth rate μ , h^{-1}
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meyenburg69phd_tab6
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rieger83_fig1_glc10
rieger83_fig1_glc5
lei01_fig3
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mian74
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vanmaris01_fig1
vanhoek98_tab1
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diderich99_fig1
P, h
growth rate μ , h^{-1}
heinzle83_fig6
heinzle83_fig6
heinzle83_fig6
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porro88_fig4_high
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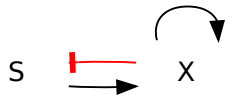
straessle89_fig9_exp2
straessle89_fig9_exp3
straessle89_fig9_exp4
murray01_fig5a
slavov14arxive_fig1c_Glc03
slavov14arxive_fig1c_Glc04
slavov14arxive_fig1c_Glc08
slavov14arxive_fig1c_Glc16
adams03_fig5b
birol00_fig5
mean cluster expression
inferred growth rate μ , h^{-1}
A, r2=0.81
AB, r2=0.78
B, r2=0.3
B.C, r2=0
C, r2=0.01
B.D, r2=0.39
D, r2=0.67

1.2 Inherent Feedbacks of Growth



$$\begin{aligned} \frac{\mathrm{d}S}{\mathrm{d}t} &= \phi(S_{in} - S) - \frac{\mu}{y}X \\ \frac{\mathrm{d}X}{\mathrm{d}t} &= (\mu - \phi)X \end{aligned} \tag{1}$$

where both, the yield $y \equiv \frac{\Delta X}{-\Delta S}$ and the growth rate μ are functions $f(S)$ of the substrate concentration. Depending on the sign of $\frac{df}{dS}$, we can see the general feedback structure of cellular growth in the Jacobi matrix.

Note the positive feedback of X on itself if $\mu > \phi$, and, if $\frac{df}{dS} > 0$ as e.g. for the Monod equation $\mu = \mu_{max}\frac{S}{S+K}$, the negative feedback between X and S:

$$J = \begin{pmatrix} & S & X \\ S & -\frac{d\mu}{dS}\frac{X}{y} - \phi & -\frac{\mu}{y} \\ X & \frac{d\mu}{dS}X & \mu - \phi \end{pmatrix} = \begin{pmatrix} & S & X \\ S & - & - \\ X & + & +/- \end{pmatrix} \tag{2}$$

Cooperativity of substrate usage, e.g. $\mu = \mu_{max}\frac{S^2}{S^2+K^2}$ or variable yield, e.g. $y = a + b \cdot S$, may already lead to multistability or oscillations [25, 26, 27, 28].

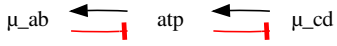
A positive dependence of the yield on substrate may represent the lower costs required for transporters at high glucose concentrations, where more energy is left for growth [28]. These models can be considered as early versions of current "ressource allocation" problems. The observed oscillations, however, had the wrong relation to dilution rate, i.e., period increased with increasing dilution rate ϕ, while in reality periods decrease with ϕ.

where is this from? heinzle doesnt mention transporters

Yeast cells switch to fermentation at high growth rates and this is accompanied by a severe drop in yield [1, 11, 17, 12], induced by high glucose concentrations, and involves differential expression of budding yeast's abundant glucose transporters [29, 30]. Thus, we should test the effects of an alternative non-monotonous yield function, where at high substrate concentration yield decreases. Could this yield correct period-growth rate relations? Could it cause bistability? How does variation of yield at a given dilution rate change the period?

2 Anabolism vs. Catabolism: explicit yield

The feedbacks of cellular growth are here extended by the fundamental feedbacks of metabolism, where intermediates, *e.g.*, nucleotide-derived coenzymes [31] or membrane potential, relay free energy from catabolic to anabolic reactions.



$$\begin{aligned}
\frac{dX}{dt} &= (\mu_{ab} - \phi)X \\
\frac{dS}{dt} &= \phi(S_{in} - S) - (\mu_{ab} + \mu_{cd})X \\
\frac{datp}{dt} &= (n_{cd}\mu_{cd} - n_{ab}\mu_{ab} - \mu_m)\frac{C_c}{V_c} - \mu_{ab}atp \\
adp &= a_{\text{tot}} - atp
\end{aligned} \tag{3}$$

where C_c and V_c are the cellular carbon content and volumes.

$$\begin{aligned}
\mu_{ab} &\equiv f(S, atp) \\
\mu_{cd} &\equiv f(S, adp) \\
n_{cd} &\equiv f(S)
\end{aligned} \tag{4}$$

where $f(x)$ are positive monotonous functions of x. Here, "yield" is represented by the catabolic and anabolic ATP stoichiometries n_{cd} and n_{ab}. Their dependencies on substrate and product concentrations, or cellular regulation (see section 2.1) can reflect differential costs of transport, catabolic or anabolic machineries. Specifically, the yield function can reflect mixtures of fermentative (low n_{cd}) and respiratory (high n_{cd}) catabolism. Coupling n_{ab} to n_{cd}, such that n_{ab} decreases with n_{cd}, can reflect the cheaper costs of fermentative biomass (less mitochondria): $n_{ab} \sim n_{cd}$.

Goals & Questions. Can this formulation of growth *via* an energy intermediate reproduce the observed relation of growth to substrate concentration (Monod's and similar equations) [32, 33, 34, 35]?

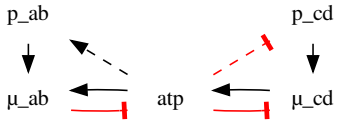
Can it oscillate?

What can we say about the interdependence of catabolic and anabolic stoichiometries, $n_{ab} \sim n_{cd}$ wrt the fermentation vs. respiration question?

Additional explicit substrate dependence in μ_{ab} could account for glucose-sensing effects [29]. It was suggested that cell volume growth in yeast depends on external glucose concentrations *via* extracellular sensors, while cell division depends on glucose influx [30]. Can we find a simple way to differentiate growth and cell division (perhaps just in result or parameter interpretation)?

2.1 Explicit Feedback

Does an additional direct feedback on rates μ by atp, beyond intrinsic balancing, have an effect on rate or substrate yield?



Can this simple homeostasis reproduce the linear relations of growth rate with the growth clusters AB and stress clusters CD (inverse to growth) that were observed in [36, 37, 38, 39]? To analyze these questions, first add non-linear dependencies of μ in equations 4, then add an abstract gene regulation of enzymes p_{AB} (μ_{ab}) and p_{CD} (μ_{cd}), where expression of AB is activated and CD repressed by atp either in parameters k (translation rate) or r (RNA level). Can we reproduce and rationalize the linear transition from oxidative to fermentative metabolism at constant growth rate but decreasing substrate [15]? For this question it may suffice to account for increasing stoichiometries n_{ab} and n_{cd} with increasing fraction of oxidative enzymes p_{cd}, or alternatively introduce a separate fermentative enzyme fraction p_{fr}. The stoichiometries n_{ab} and n_{cd} could then become function of the (relative) expression levels of these enzymes. Additional dependences of the expression rates r (transcription and translation) on substrates (S, N, O_2, products) can reflect direct regulatory effects.

$$\begin{aligned}
\frac{\mathrm{d}p_{ab}}{\mathrm{d}t} &= (k_{ab}r_{ab} - p_{ab}d_{ab})\frac{C_c}{V_c} - \mu_{ab}p_{ab} \\
\frac{\mathrm{d}p_{cd}}{\mathrm{d}t} &= (k_{cd}r_{cd} - p_{cd}d_{cd})\frac{C_c}{V_c} - \mu_{ab}p_{cd} \\
\frac{\mathrm{d}p_{fr}}{\mathrm{d}t} &= (k_{fr}r_{fr} - p_{fr}d_{fr})\frac{C_c}{V_c} - \mu_{ab}p_{fr}
\end{aligned} \tag{5}$$

$$\begin{aligned}
k_{ab}, r_{ab}, d_{ab} &\equiv f(atp, p_{ab}) \\
k_{cd}, r_{cd}, d_{cd} &\equiv f(adp, p_{ab}) \\
k_{fr}, r_{fr}, d_{fr} &\equiv f(S, adp, p_{ab}) \\
\mu_{ab} &\equiv f(S, atp, p_{ab}) \\
\mu_{cd} &\equiv f(S, adp, p_{cd}) \\
n_{cd}, n_{ab} &\equiv f(\frac{p_{cd}}{p_{cd} + p_{ab}})
\end{aligned} \tag{6}$$

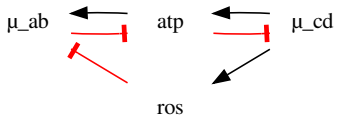
2.1.1 Trade-off Constraint

To explicitly account for and analyze ressource allocation, include the atp and S costs of synthesis rates. Or simpler, use sum of all proteins $\sum p_i =$ const. as a constraint? Or can that be a post-analysis of the model?

2.2 Futile Cycles, Storage and Biomass Recycling

Account for an "aged" non-productive biomass fraction Y (e.g. lipids and proteins) damaged by a toxic metabolite R (e.g. ROS), and allow catabolization of Y and removal of R by the maintenance reaction μ_m. Alternatively, the same model may also reflect storage metabolism; the dependence on R can be simply omitted.

or not, that would actually break C-mol balance



$$\begin{aligned} S + n_{cd} ADP &\rightarrow n_{cd} ATP + n_{cr} R && \mu_{cd} X \\ Y + n_{xd} ADP &\rightarrow n_{xd} ATP + n_{cr} R && \mu_{xd} Y \\ ATP + n_{mr} R &\rightarrow ADP && \mu_m X \\ X + n_{xr} R &\rightarrow Y && \mu_{xr} X \end{aligned} \tag{7}$$

plus additional outflow of Y and R with ϕ, and

$$\begin{aligned} \frac{dX}{dt} &= (\mu_{ab} - \mu_{xr} - \phi) X \\ \frac{dS}{dt} &= \phi(S_{in} - S) - (\mu_{ab} + \mu_{cd}) X \\ \frac{datp}{dt} &= (n_{cd}\mu_{cd} + n_{xd}\mu_{xd} - n_{ab}\mu_{ab} - \mu_m)\frac{C_c}{V_c} - \mu_{ab} atp \\ \frac{dY}{dt} &= \mu_{xr} X - (\mu_{xd} + \phi) Y \\ \frac{dR}{dt} &= n_{cr}(\mu_{cd} + \mu_{xd}) X - (n_{xr}\mu_{xr} + n_{mr}\mu_m) X - \phi R \end{aligned} \tag{8}$$

where $n_{xd} < n_{ab}$, since recovery of ATP must be smaller then investment. The stoichiometries n_{cr} and n_{mr} can, *e.g.*, reflect quality of oxidative biomass (mitochondria) and costs of R (maintenance). One may assume that a lower yield in catabolism also implies a lower yield in the toxic metabolite R, i.e. $n_{cr} \sim n_{cd}$.

Goals & Questions. In contrast to flux optimization scenarios and perhaps counterintuitively, carbohydrate storage, essentially a futile cycle, is used especially in slowly growing cells on low substrate concentration [40].

Recently, the futile cycle of trehalose metabolism was shown to buffer imbalances in anabolic and catabolic reactions [41].

During reductive phase CO2 comes from glucose, during oxidative phase, CO2 is derived from lipids and proteins.

Can the futile cycles of storage or recycling of damaged biomass be rationalized?

storage and futile cycles need an explicit model

2.3 Fermentation *vs.* Respiration

To further account for Slavov's data [15] on the transition from respiration to fermentation in batch culture, while maintaining a constant growth rate, or the rate vs. yield question [42], we can add an alternative catabolic path to ethanol. Such models have often been analyzed and yielded rich dynamics [43].

$$\begin{aligned} \mathrm{S} + 2\mathrm{ADP} &\rightarrow 2\mathrm{CO_2} + 2\mathrm{ATP} + 2\mathrm{E} && \mu_{fr}X \\ \mathrm{S} + 6\mathrm{O_2} + 29.8\mathrm{ADP} &\rightarrow 6\mathrm{CO_2} + 29.8\mathrm{ATP} && \mu_{go}X \\ \mathrm{E} + 3\mathrm{O_2} + 12.9\mathrm{ADP} &\rightarrow 3\mathrm{CO_2} + 12.9\mathrm{ATP} && \mu_{eo}X \end{aligned} \tag{9}$$

After converting above stoichiometries to C-mol:

$$\begin{aligned} \frac{dS}{dt} &= \phi(S_{in} - S) - (\mu_{ab} + \mu_{fr} + \mu_{go})X \\ \frac{dE}{dt} &= \phi(E_{in} - E) + (n_{E,fr}\mu_{fr} - \mu_{eo})X \\ \frac{dO_2}{dt} &= \kappa_{O_2}(O^*_{2,in} - O_2) - (n_{O_2,eo}\mu_{eo} + n_{O_2,go}\mu_{go})X \\ \frac{dCO_2}{dt} &= \kappa_{CO_2}(CO^*_{2,in} - CO_2) + (n_{CO_2,fr}\mu_{fr} + n_{CO_2,go}\mu_{go} + n_{CO_2,eo}\mu_{eo})X \\ \hline \frac{dX}{dt} &= (\mu_{ab} - \phi)X \\ \frac{datp}{dt} &= (n_{fr}\mu_{fr} + n_{go}\mu_{go} + n_{eo}\mu_{eo} - n_{ab}\mu_{ab} - \mu_m)\frac{C_c}{V_c} - \mu_{ab}atp \\ adp &= 1 - atp \end{aligned} \tag{10}$$

with gas and ethanol stoichiometries: $n_{O_2,eo} = n_{CO_2,eo} = 1.5$, $n_{O_2,go} = n_{CO_2,go} = 1$, $n_{E,fr} = n_{CO2,fr} = \frac{4}{6}$, and catabolic ATP stoichiometries: $n_{eo} = 6.45$, $n_{go} = 4.97$, $n_{fr} = \frac{2}{6}$.

Fermentation *vs.* Respiration: the Pyruvate Node.

$$\begin{aligned} \mathrm{S} + 2\mathrm{ADP} + 2\mathrm{NAD^+} &\rightarrow 2\mathrm{P} + 2\mathrm{ATP} + 2\mathrm{NADH} && \mu_{gl}X \\ \mathrm{P} + \mathrm{NADH} &\rightarrow \mathrm{E} + \mathrm{NAD^+} + \mathrm{CO_2} && \mu_{fr}X \\ \mathrm{E} + 2\mathrm{NAD^+} + \mathrm{ATP} &\rightarrow \mathrm{A} + 2\mathrm{NADH} + \mathrm{ADP} && \mu_{eo}X \\ \mathrm{P} + \mathrm{NAD^+} &\rightarrow \mathrm{A} + \mathrm{NADH} + \mathrm{CO_2} && \mu_{ox}X \\ \mathrm{A} + 3.6\mathrm{NAD^+} + \mathrm{ADP} &\rightarrow 3.6\mathrm{NADH} + \mathrm{ATP} + 2\mathrm{CO_2} && \mu_{tc}X \\ \mathrm{NADH} + 2.3\mathrm{ADP} + \frac{1}{2}\mathrm{O_2} &\rightarrow \mathrm{NAD^+} + 2.3\mathrm{ATP} && \mu_{cd}X \end{aligned} \tag{11}$$

where S is now glucose, P is pyruvate, E ethanol and A Acetyl(-CoA). In μ_{eo} and μ_{po}, which represent TCA cycle oxidation of ethanol and pyruvate, we count $\mathrm{QH_2}$ as 0.6 NADH, assuming to yield 2.3 ATP per NADH and 1.4 ATP

per QH_2 in the electron transport chain [44]. We further count the GTP produced in TCA oxidation of Acetyl-CoA as ATP. Studying rate/yield effects again can be represented in a dependence of n_{ab} on the relative expression levels of oxidative *vs.* fermentative enzymes. TODO: simplify the pyruvate $\leftrightarrow$ Acetyl-CoA interconversions to an intracellular intermediate C:

split Glc phosph from glycolysis; introduce storage and overall gluconeogenesis from AcCoA

$$\begin{aligned}
\frac{dS}{dt} &= \phi(S_{in} - S) - \mu_{gl} X \\
\frac{dC}{dt} &= (\mu_{gl} + \mu_{eo} - \mu_{fr} - \mu_{ab} - \mu_{ox}) X \\
\frac{dE}{dt} &= \phi(E_{in} - E) + (\mu_{fr} - \mu_{eo}) X
\end{aligned} \tag{12}$$

Intracellular concentrations. As in [12], We could assume constant intracellular pH of ca. 7 and a pH-dependent equilibrium between intracellular and extracellular concentrations of pyruvate, and use only the intracellular fraction in rate laws. However, [4] used the same equations and intracellular and extracellular acetate concentrations to show varying intracellular pH.

2.4 qCO2 Model : Feedback on Respiration?

$$
\begin{aligned}
\mathrm{C_6} + 2\mathrm{ADP} &\rightarrow 2\mathrm{C_3} + 2\mathrm{ATP} && \mu_{gl}X \\
\mathrm{C_3} &\rightarrow \mathrm{C_2} + \mathrm{CO_2} && \mu_{fr}X \\
\mathrm{C_2} + \mathrm{ATP} &\rightarrow \frac{2}{3}\mathrm{C_3} + \mathrm{ADP} && \mu_{eo}X \\
\mathrm{C_3} + \mathrm{n_{cd}ADP} &\rightarrow 3\mathrm{CO_2} + \mathrm{n_{cd}ATP} && \mu_{ox}X \\
\mathrm{C_3} + \mathrm{CO_2} + \mathrm{ATP} &\rightarrow \mathrm{C_{tca}} + \mathrm{ADP} && \mu_{tca}X \\
\mathrm{xC_3} + \mathrm{yCO_2} + \mathrm{n_{ac}ATP} &\rightarrow \mathrm{zAMP} + \mathrm{n_{ac}ADP} && \mu_{aicar}X \\
\mathrm{AMP} + \mathrm{ATP} &\rightarrow 2\mathrm{ADP} && \mu_{ak}X \\
\mathrm{C_{tca}} + \mathrm{m_{ab}ATP} &\rightarrow \mathrm{X} + \mathrm{n_{ab}ADP} && \mu_{ab}X
\end{aligned}
\tag{13}
$$

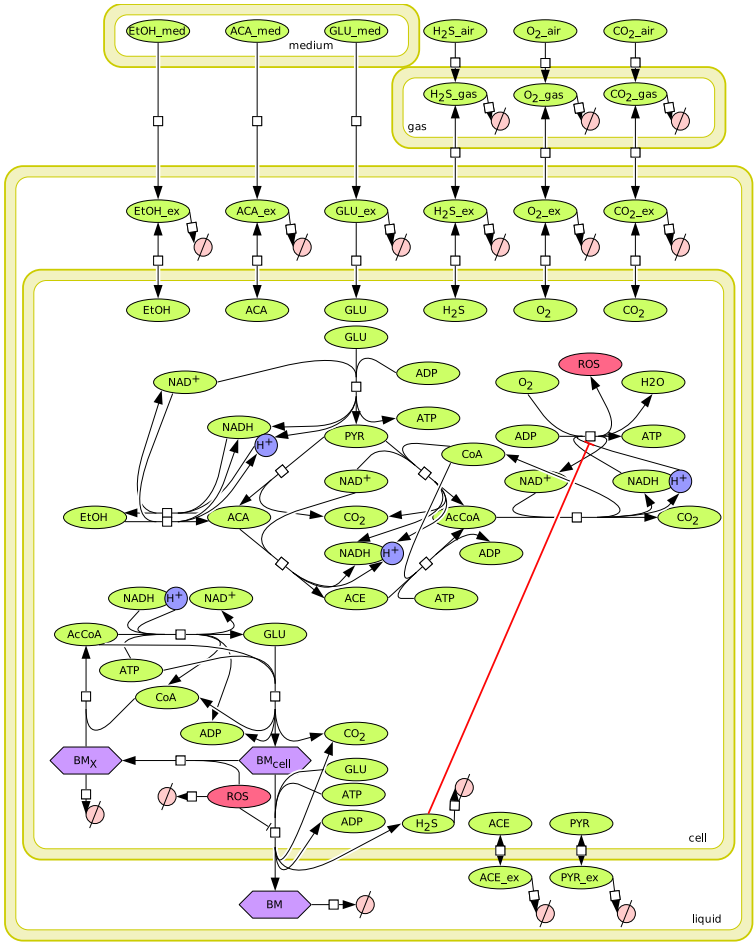
2.5 Explicit Intracellular Dynamics

This model differentiates intra- and extracellular metabolites to account for, e.g., active glucose transport, storage and overflow metabolism.

An average cell is modelled and reactor-level fluxes of imported and exported metabolites, are scaled by cell number n calculated from the current biomass concentration. For example:

$$\begin{aligned} n\ Glc_{ex} &\rightarrow Glc \\ Glc &\rightarrow X_{in} \\ X_{in} &\rightarrow n\ X \end{aligned} \tag{14}$$

where Glc_{ex} and X are culture concentrations of glucose and biomass, and Glc and X_{in} are concentrations inside the average cell, all in C-Mol. The stoichiometry to scale from one average to all cells is calculated simply as $n = X/C_c$, where C_c is the carbon content per cell (C-mol/cell).



based on tataProject model `corecarbon_v4_detailed.sbml`

2.6 Plasmid Replication & Gene Expression

As a replicative entity a plasmid's growth is auto-catalytic and thus exponential and limited by substrates (nucleotide-triphosphates, polymerases). A fluorescent protein with intracellular concentration f in mol/L is expressed from this plasmid.

$$\begin{aligned}
\frac{\mathrm{d}X}{\mathrm{d}t} &= (\mu_{ab} - \phi)X \\
\frac{\mathrm{d}S}{\mathrm{d}t} &= \phi(S_{in} - S) - (\mu_{ab} + \mu_{cd})X \\
\frac{\mathrm{d}atp}{\mathrm{d}t} &= m_{cd}\mu_{cd} - m_{ab}\mu_{ab} - m_m\mu_m - m_p k_p - m_f k_f p - \mu_{ab} atp \\
adp &= axp_{tot} - atp \\
\frac{\mathrm{d}p}{\mathrm{d}t} &= (k_p - \mu_{ab})p \\
\frac{\mathrm{d}f}{\mathrm{d}t} &= k_f p - (d_f + \mu_{ab})f
\end{aligned} \tag{15}$$

Reporter variables - to fit to data:

$$\begin{aligned}
OD &= f_{od} X \\
FL &= f_{fl} f X
\end{aligned} \tag{16}$$

Stoichiometries:

$$m_i = n_i \frac{C_c}{V_c} \tag{17}$$

The ATP stoichiometries m_i take units and values that depend on the units of biomass X and substrate S and map from their culture-level concentrations to intracellular concentrations. For example, if X and S are modelled in C-mol liter^{-1}, then $m_i = n_i \frac{C_c}{V_c}$, where n_i is the metabolic ATP stoichiometry of growth or catabolism with respect to substrate S, C_c is the average carbon content of cells in mol and V_c an average cell volume in liter, and atp is the intracelluler concentration of ATP in mol liter^{-1}. See Appendix A for details.

Rates:

$$\begin{aligned}
\mu_{ab} &\equiv f(S, atp) \\
\mu_{cd} &\equiv f(S, adp) \\
k_p &\equiv f(S, atp) \quad = \frac{1}{1 + \frac{S}{K_{p,S}}} \frac{atp}{atp + K_{p,atp}} \\
k_f &\equiv f(S, atp) \quad = \frac{S}{S + K_{f,S}} \frac{atp}{atp + K_{f,atp}}
\end{aligned} \tag{18}$$

The growth (=replication) rate of plasmid p is inhibited by substrate S and/or atp/adp ratio to reflect stationary state boost of high-copy plasmids (or use substrate inhibition for S?).

2.7 Complex Substrates

To study effects of several substrates, add e.g. nitrogen flux in liquid (dilution, ϕ) as a requirement for μ_{ab} or oxygen/carbon dioxide flux in gas (aeration, $\kappa \equiv f(k_L a)$ [45]) as a requirement for μ_{cd}. The latter can also be used (without kinetic effects) to calibrate the model to offgas data from bioreactors.

E.g. for growth on ethanol:

$$\begin{aligned} S + n_{an}N + n_{ab}ATP &\rightarrow X + n_{ab}ADP \quad && \mu_{ab}X \\ S + n_{O_2}O_2 + n_{cd}ADP &\rightarrow CO_2 + n_{cd}ATP \quad && \mu_{cd}X \end{aligned} \tag{19}$$

where, e.g., $n_{O_2} = 1.5$ and $n_{cd} = 6.45$ would be the oxygen and ATP stoichiometries per carbon molecule of ethanol oxidation.

$$\begin{aligned} \frac{dN}{dt} &= \phi(N_{in} - N) - n_{an}\mu_{ab}X \\ \frac{dO_2}{dt} &= \kappa_{O_2}(O^*_{2,in} - O_2) - n_{O_2}\mu_{cd}X \\ \frac{dCO_2}{dt} &= \kappa_{CO_2}(CO^*_{2,in} - CO_2) + \mu_{cd}X \end{aligned} \tag{20}$$

It may be required to include storage reactions (7) and intermediate (fixed) N species to get any interesting effects or account for data on other-than-carbon limitations as in [39]. Including non-linear dependencies of respiration on oxygen or carbon dioxide may allow faster oscillations. See [28, 43] for dynamics when including liquid and gas mass transfer.

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A Intracellular vs. Culture Level

Here, we want to align intracellular concentrations with total culture concentration. Culture level concentrations of biomass X and carbon-substrate S are modeled in carbon mol liter^{-1}.

A.1 From Cell to Reactor

Consider a substance P produced and degraded inside cells, *e.g.*, a a RNA or a protein. It's intracellular concentration p (mol liter^{-1}) changes as a sum of production, degradation and cell growth:

$$\frac{\mathrm{d}p}{\mathrm{d}t} = k - (d + \mu)p \; . \tag{21}$$

With the average cellular volume V_c, a cell contains $p \cdot V_c$ mol of substance P. With N as the total number of cells in the culture, and V_R the total culture volume, the concentration of P in the total culture volume is:

$$P = p\frac{V_c\, N}{V_R} \tag{22}$$

Now, we model biomass not in terms of cell number N, but as the number of carbon atoms fixed in biomass. Our main biomass variable is X the, concentration of biomass carbon in the culture, in mol liter^{-1}. With an average carbon content of a cell C_c (in mol cell^{-1}), we can calculate the total number of cells in the culture as $N = \frac{X}{C_c} V_R$, and replacing this in above equation:

$$P = p\, X \frac{V_c}{C_c} \tag{23}$$

We assume that the average cell volume V_c and the average carbon content per cell C_c are constant. Only p and X change over time, and using the product rule of differentation and for simplicity the dot-notation, $\dot{p} = \frac{\mathrm{d}p}{\mathrm{d}t}$, we get:

$$\dot{P} = \dot{p}\, X \frac{V_c}{C_c} + \dot{X}\, p \frac{V_c}{C_c} \tag{24}$$

Replacing $\dot{p}$ with equation 21, the exponential growth $\dot{X} = (\mu - \phi)X$, and $p = P\frac{C_c}{X\, V_c}$ yields:

$$\frac{\mathrm{d}P}{\mathrm{d}t} = k\, X \frac{V_c}{C_c} - (d + \phi)P \tag{25}$$

where the intracellular production is now scaled by the total biomass in the culture, and P leaves the system with culture dilution rate ϕ, replacing the growth rate μ in the inital equation eq. 21.

A.2 From Wells to Cells

Cell Growth. Cells X growing in one well are converting substrate S to more cells and catabolic product P. The overall reaction is:

$$\text{S} \rightarrow \text{y X} + \text{(1-y) P}$$

The yield factor $y \equiv \frac{\Delta X}{-\Delta S}$ is the fraction of S (*e.g.* glucose or a mix of substances in the case of LB medium) that is actually converted to cells, *i.e.*, used as a carbon source for biomass formation. The remaining consumption of S (1-y) is due to the dual nature of S as both, carbon source and as energy source, and 1-y is also the stoichiometry of catabolic products P (*e.g.*, acetate, CO_2). Note, that the yield factor has units to convert between the units of X and S, *e.g.* mol^{-1}, to convert from mol (S) to cell number (X). **How can we estimate the yield y?**

Life is auto-catalytic, *i.e.* above reaction is catalyzed by X, leading to exponential growth. The growth of cells (X in cells liter^{-1}) and decrease of substrate S (mol liter^{-1}) can be described as:

$$\begin{aligned} \frac{\mathrm{d}X}{\mathrm{d}t} &= \mu X \\ \frac{\mathrm{d}S}{\mathrm{d}t} &= -\frac{1}{y}\mu X \end{aligned} \tag{26}$$

where μ is the specific growth rate (h^{-1}) that depends on the available substrate S, *e.g.* *via* Monod's equation $\mu = \mu_{max}\frac{S}{S+K}$. Wells are inoculated at time t_0 to an initial cell concentration X_0 into growth medium with initial substrate concentration S_0, and X_0 and S_0 are also known as the initial conditions of above ordinary differential equation (ODE) system.

Note, that simple modifications of this model, *e.g.* Hill constants in the Monod equation or a yield factor that itself depends on S (**what could that mean?**) can yield multistable or oscillatory solutions.

Fluorescent Protein Production. Consider a substance F that is produced and degraded within cells, *e.g.* a fluorescent protein. We express it as a concentration (mol liter^{-1}) in the total culture culture volume. F will accumulate at culture level as:

$$\frac{\mathrm{d}F}{\mathrm{d}t} = K(?) - dF \tag{27}$$

where d is the substance's degradation rate (s^{-1}), and K is its production rate in mol liter^{-1} s^{-1}. We know F is produced inside cells, what what does that mean on culture level? We know that K must be directly proportional to the cell number, $K \sim X$, **but how can we map from intracellular rates to total culture production rate?**

Intracellular Concentration and Dynamics. For modeling intracellular reactions, such as dynamic regulation of gene expression, we need its intracellular concentration f.

V_c is the volume of one average cell (liter). Multiplied by the cell number per liter X, we get a scaling factor $v_f = XV_c$, the dimensionless intracellular volume fraction of the culture, *i.e.* the fraction of the culture volume that is occupied by cells (neglecting cell walls).

The substance's intracellular concentration f (mol liter^{-1}) and its change over time, here in short notation $\dot{f} = \frac{\mathrm{d}f}{\mathrm{d}t}$, calculated by the product and inverse function rules of differentiation, are:

$$\begin{aligned} f =& \frac{F}{XV_c} \\ \dot{f} =& \dot{F}\frac{1}{XV_c} - \dot{X}\frac{F}{X}\frac{1}{XV_c} \\ \dot{f} =& \dot{F}\frac{1}{v_f} - \dot{X}\frac{F}{X}\frac{1}{v_f} \end{aligned} \tag{28}$$

Replacing $\dot{F}$ (eq. 27), the exponential growth $\dot{X}$ (from eq. 26), and $F = fXV_c = f\,v_f$ yields:

$$\begin{aligned} \dot{f} =& (K - d\,f\,v_f)\frac{1}{v_f} \quad -\mu X\frac{f v_f}{X}\frac{1}{v_f} \\ =& \frac{K}{v_f} - d\,f - \mu\,f \\ =& \frac{K}{v_f} - (d+\mu)f \end{aligned} \tag{29}$$

We see that $K = kXV_c$ is simply a scaled version of the actual cellular production rate k in mol liter^{-1} s^{-1}.

We can now combine eq. 26 and 29 into a relatively simple multiscale ODE model.

$$\begin{aligned} \frac{\mathrm{d}X}{\mathrm{d}t} &= \mu X \\ \frac{\mathrm{d}S}{\mathrm{d}t} &= -\frac{1}{y}\mu X \\ \frac{\mathrm{d}f}{\mathrm{d}t} &= k - (d+\mu)f \end{aligned} \tag{30}$$

which can easily be extended to account for intracellular regulatory processes, *e.g.*, **activation of f production by an inducer and modulation by a riboswitch r, or expression of both from a plasmid (which itself increases exponentially within cells), where the plasmid copy number p can be accounted for. And how can we account for the effect of induced gene expression on growth rate?**

A.3 Growth Models

$$X(t) = A(1 + \nu e^{1+\nu+\frac{\mu}{A}(1+\nu)^{1+\frac{1}{\nu}}(\lambda-t)})^{-\frac{1}{\nu}}$$

A.4 Stoichiometric Coupling between Cells and Reactor

To account for intracellular metabolic reactions, *i.e.*, explicitly couple the metabolism of S into biomass X and catabolic products P to intracellular processes, is slightly more involved.

Above, we introduced a simple trick to map between intracellular and total culture concentration, but the intracellular reaction was uncoupled from culture level variables. Now, we want to couple intracellular metabolic reactions to culture level variables, such as substrate or biomass concentrations. We consider a simple growth model, where the conversion of substrate to biomass is modelled *via* an intracellular intermediate.

Let's first consider the overall growth reaction, where a carbon substrate S, *e.g.* glucose, is converted partially to biomass X and the other part to some product P. The fraction of S that is converted to biomass X is the so-called yield factor $y \equiv \frac{\Delta X}{-\Delta S}$, similar to a stoichiometry in chemical reactions. The rest of S is converted to product:

$$\text{S} \rightarrow y\ \text{X} + (1\text{-}y)\ \text{P}$$

We want to know the rate of biomass production (growth), and thus divide by the yield factor:

$$\tfrac{1}{y}\ \text{S} \rightarrow \text{X} + (\tfrac{1}{y}\text{-}1)\ \text{P}$$

This reaction is catalyzed by X, *i.e.*. life is auto-catalytic. If S is not limiting X is produced with the rate μX, which is the definition of exponential growth:

$$\begin{aligned} \frac{dX}{dt} &= \mu X \\ \frac{dS}{dt} &= -\frac{1}{y}\mu X \end{aligned} \tag{31}$$

Note that if both S and X are in the same units, *e.g.*, mol of carbons, $y < 1$ and there is always more S consumed then X produced. The actualy yield factor y depends on many things and varies with growth rate μ. Where does it come from and what does it mean mechanistically? The most general explanation of yield is thermodynamic. A unit of biomass X has a lower or equal entropy then a unit of substrate S. Additionaly entropy increase must compensate for the decrease in entropy during conversion of S to X, and this is obtained catalyzing conversion of additional S to higher entropy molecules, *e.g.*, oxidization of glucose to carbon dioxide.

which paper was it again? what is the argument or yield at equal?

The compensating entropy increase of catabolic reactions is not directly coupled to anabolic reactions. Instead life universally employs chemical intermediates, analogous to currencies. In glycolysis, the free energy stored in the substrate glucose is transferred to the high-energy bonds of adenosine triphosphate (ATP). Another central cellular intermediate of the harvested free energy is the electrochemical potential generated across all cellular membranes. This electrochemical potential is again converted to ATP by ATP synthetase in both respiratory and photosynthetic electron transport chains.

fundamental arguments for intermediates?

With this knowledge, we can now more explicitly model above yield factor. While many metabolic intermediates exist, for convenience, let's focus on the most central, ATP.

$$\mathrm{S} + n_{cd}\ \mathrm{ADP} \rightarrow n_{cd}\ \mathrm{ATP} + \mathrm{P}$$

B Specific Models

B.1 Competition between Respirer and Fermenter

As suggested by Willi. Co-existence of a purely fermenting species X_1 and a purely oxidative species X_2, which can also oxidize the ethanol produced by the former. For this the model in section 2.3 is split into two species.

$$\begin{array}{r}
\frac{dS}{dt} = \phi(S_{in} - S) - (\mu_{ab_1} + \mu_{fr})X_1 - (\mu_{ab_2} + \mu_{go})X_2 \\
\frac{dE}{dt} = \phi(E_{in} - E) + n_{E,fr}\mu_{fr}X_1 - \mu_{eo}X_2 \\
\frac{dO_2}{dt} = k_L a_{O_2}(O^*_{2,in} - O_2) - (n_{O_2,eo}\mu_{eo} + n_{O_2,go}\mu_{go})X_2 \\
\hline
\frac{dX_1}{dt} = (\mu_{ab_1} - \phi)X_1 \\
\frac{datp_1}{dt} = (n_{fr}\mu_{fr} - n_{ab_1}\mu_{ab_1} - \mu_{m_1})\frac{C_c}{V_c} - \mu_{ab_1}atp_1 \\
adp_1 = 1 - atp_1 \\
\hline
\frac{dX_2}{dt} = (\mu_{ab_2} - \phi)X_2 \\
\frac{datp_2}{dt} = (n_{go}\mu_{go} + n_{eo}\mu_{eo} - n_{ab_2}\mu_{ab_2} - \mu_{m_2})\frac{C_c}{V_c} - \mu_{ab_2}atp_2 \\
adp_2 = 1 - atp_2 \\
\hline
\end{array} \tag{32}$$

B.1.1 Rate equations

$$\begin{aligned}
\mu_{fr} &\equiv c(S, adp_1) \\
\mu_{go} &\equiv c(S, adp_2, O_2) \\
\mu_{eo} &\equiv c(E, adp_2, O_2) \\
\mu_{ab_1} &\equiv g(S, atp_1) \\
\mu_{ab_2} &\equiv g(S, atp_2)
\end{aligned} \tag{33}$$

The catabolic functions c can be simple Michaelis-Menten kinetics, while the growth functions g can additionally account for glucose-sensing effects, as e.g. in the phenomenological growth function observed by Youk and van Oudenaarden [29] or ethanol inhibition of growth. The pure Monod-function with simple dependence of growth on substrate concentration should arise from the dependence on ATP.

B.1.2 Parameters

The main source of below parameter calculations is file `yeast.carbon.v1_notes.txt`. Note that stoichiometries are defined in C-mol or for non-carbon metabolites such as ATP and O_2 in mol / C-mol.

Gas Transfer. See [45], for now using value of 190 µM measured by DBM.

Biomass in C-mol. Korhola and Edelman reported 0.46 (g C)/(g DW) [46]. Using MW 12.0107 (g C)/(mol C), we get 0.03829918 C-mol/(g DW), and for DBM values of 8 (g DW)/L and 5e11 cells/L, we get 1.6e-11 (g DW)/cell. Combining these values, we obtain C_c=6.127869e-13 C-mol/cell.

Using a cell diameter of d=4.7 µm, we get a cellular volume of $(d/2)^3 * 4/3 * pi$, *i.e.*, V_c=5.43616e-14 L.

Catabolic Stoichiometries. The catabolic stoichiometries were calculated per C-mol of glucose and ethanol, (where a glucose molecule has 6 C-mol and ethanol 2 C-mol) from the known stoichiometries of their fermentation and oxidation pathways, and assuming a yield of 2.3 ATP per NADH and 1.4 ATP per reduced quinone in the electron transport chain [44]. Section 2.3 gives explicit the reactions.

TODO: compare these values with those given in [47] "PO ratio of 1.09 mol of ATP/mol of O (95% confidence interval 1.07-1.11)"

TODO: check if this is all correct. Note: the ATP yield per carbon seems to be higher for ethanol, which is compatible with the increased C-mol yield on ethanol [48].

Anabolic Stoichiometries. Lagunas reported 9, 7 and 3 g DW/mol ATP in glucose, galactose and ethanol cultures [49], Verduyn *et al.* reported 16 g biomass per mol ATP [...] "at a dilution rate of 0.10/h for anaerobic growth" [50]. "The costs adopted in the yeast metabolic network model of Mo et al. [51], obtained by fitting FBA results to biomass yield measured in the aerobic, glucose-limited continuous culture experiment of Verduyn et al. ([52, 53]" were 59.276 mmol ATP/g DW for growth, i.e., 16.87023 g DW/mol ATP, and 1.0 mmol ATP/(g DW*h) for maintenance costs.

We start with 9 g DW/mol ATP [49] for aerobic growth, and using above value for C-mol/g DW we obtain $n_{ab,2}$=1/(0.03829918*9) = 2.901136 (mol ATP)/(C-mol DW); and with 16 g DW/mol ATP [50] for fermentation, we obtain $n_{ab,1}$=1/(0.03829918*16) = 1.631889 (mol ATP)/(C-mol DW).

check there; wher is this quote from?

Maintenance Rate. For maintenance costs, we take the 1 mmol ATP/(g DW*h) from [51] and in C-mol obtain for μ_m=0.001/0.03829918=0.02611022 (mol ATP)/(C-mol*h).

An empirical equation has been calculated by Tijhuis *et al.* [54], as given in the review by Heijnen [55]:

$\mu_m = 4.5e^{69000/R*(1/298-1/T)}$

in kJ Gibbs energy per hour per C-mol biomass. For growth at T=30°C this yields $\mu_m = 7.1$.

calculate this for ATP

Again from the Heijnen lab, ref. [47] reports a value of "a k factor of 0.415 mol of ATP/C-mol of biomass (0.385-0.445)" for maintenance at dilution rate D=0.1h^{-1}. This would give a value (TODO: check) of 0.0415 (mol ATP)/(C-mol*h), thus not too far away from the value obtained above!

Maximal Rates. Based on measured maximal specific rates qO_2 of 5 mmol/(g*h) [3], and using above value for C-mol/(g DW) we get = 131 (mmol O_2)/(C-mol*h). Using above O_2 stoichiometries of 1.5 (mol O_2)/(C-mol ethanol) and 1 (mol O_2)/(C-mol glucose), we can calculate catabolic/respiratory substrate rates for glucose and ethanol of 0.131 and 0.087 (C-mol glucose|ethanol)/(C-mol*h), respectively.

Heijnen and co-workers suggested that the maximal specific respiration rate is limited by membrane space, where smaller cells have a larger surface/volume to ratio, and gave an empirical maximal rate of electron flux per biomass

$q^{el}_{max} = 3e^{69000/R*(1/298-1/T)}$

in (mol electrons)/(C-mol*h) [55]. For growth at T=30°C this yields $q^{el}_{max} = 4.75$ Two electrons are transferred from NADH to $\frac{1}{2}$ O_2, thus 4 electrons per O_2, this would give a maximal rate of $q^{O_2}_{max}$=1.19 mol O_2)/(C-mol*h), a factor 10 higher then the rate used above. The highest literature value for qO_2 is found in [12] with 12 mmol/(g*h) or 0.3

check this calc. and investigate further

Main Open Question: How to represent a common μ_{max} for respiration of ethanol and glucose?

parameter	value	unit	ref.
S_{in}		C-Mol	
E_{in}		C-Mol	
ϕ	0.1	h^{-1}	reference value
$k_L a_{O_2}$	100	h^{-1}	TODO [45]
$O^*_{2,in}$	190e-6	Mol	TODO [45]
C_c	6.1e-13	C-mol/cell	[46], TODO: [48]
V_c	5.4e-14	L/cell	from cell diameter 4.7 µm
C_c/V_c	11	C-mol/L	
$n_{E,fr}$	$\frac{4}{6}$		
$\mu_{fr,max}$		h^{-1}	
K_{fr,adp_1}		Mol	
$K_{fr,S}$		C-Mol	
n_{go}	4.97	mol ATP / C-mol glucose	
$n_{O_2,go}$	1	mol O_2 / C-mol glucose	
$\mu_{go,max}$	0.13	(C-mol glucose/C-mol X)*h^{-1}	*via* $qO2_{max}$, TODO
K_{go,adp_2}		Mol	
$K_{go,S}$		C-Mol	
K_{go,O_2}		Mol	
n_{eo}	6.45	mol ATP / C-mol ethanol	
$n_{O_2,eo}$	1.5	mol O_2 / C-mol ethanol	
$\mu_{eo,max}$	0.09	(C-mol ethanol/C-mol X)*h^{-1}	*via* $qO2_{max}$, TODO
K_{eo,adp_2}		Mol	
$K_{eo,E}$		C-Mol	
K_{eo,O_2}		Mol	
n_{ab_1}	1.6	mol ATP / C-mol X	[50]
$\mu_{ab_1,max}$	0.6	h^{-1}	estimated
K_{ab_1,adp_1}		Mol	
$K_{ab_1,S}$		C-Mol	
n_{ab_2}	2.9	mol ATP / C-mol X	[49]
$\mu_{ab_2,max}$	0.6	h^{-1}	estimated
K_{ab_2,adp_2}		Mol	
$K_{ab_2,S}$		Mol	
μ_{m_1}	0.03	(mol ATP/C-mol X)*h^{-1}	[51]
μ_{m_2}	0.03	(mol ATP/C-mol X)*h^{-1}	[51]

B.1.3 Respiration and Fermentation, Nitrogen

Combine base models in sections 2.7 and 2.3.

$$
\begin{aligned}
\frac{dN}{dt} &= \phi(N_{in} - N) - n_{an}\mu_{ab}X \\
\frac{dS}{dt} &= \phi(S_{in} - S) - (\mu_{fr} + \mu_{go} + \mu_{ab})X \\
\frac{dE}{dt} &= \phi(E_{in} - E) + (n_{E,fr}\mu_{fr} - \mu_{eo})X \\
\frac{dO_2}{dt} &= \kappa_{O_2}(O^*_{2,in} - O_2) - (n_{O_2,eo}\mu_{eo} + n_{O_2,go}\mu_{go})X \\
\frac{dCO_2}{dt} &= \kappa_{CO_2}(CO^*_{2,in} - CO_2) + (n_{CO_2,fr}\mu_{fr} + n_{CO_2,go}\mu_{go} + n_{CO_2,eo}\mu_{eo})X \\
\hline
\frac{dX}{dt} &= (\mu_{ab} - \phi)X \\
\frac{datp}{dt} &= (n_{fr}\mu_{fr} + n_{go}\mu_{go} + n_{eo}\mu_{eo} - n_{ab}\mu_{ab} - \mu_m)\frac{C_c}{V_c} - \mu_{ab}atp \\
adp &= 1 - atp
\end{aligned}
\tag{34}
$$