IIA 2. Bacterial growth physiology

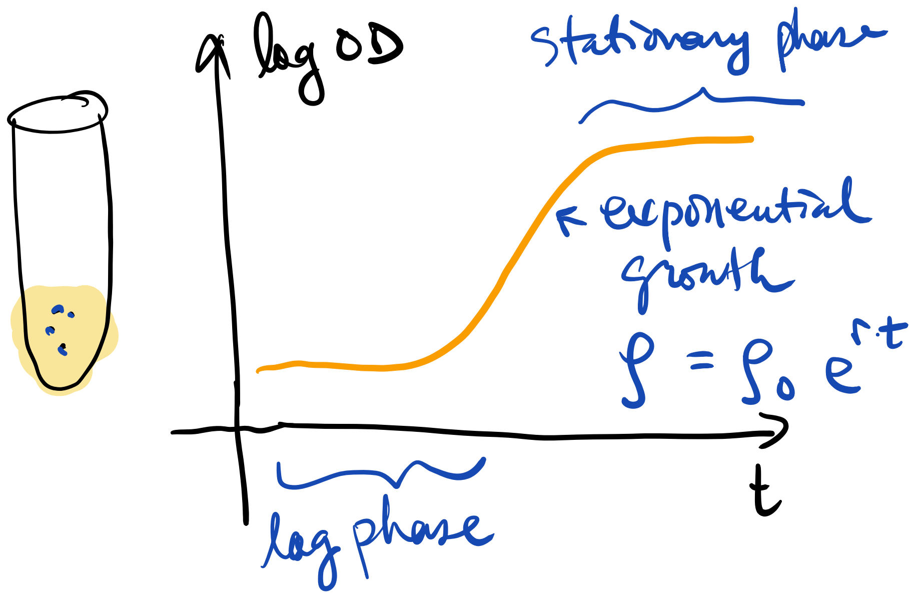
a) Overview of bacterial growth: cell + medium → cells

Medium ingredients:

- nutrients
  - C: Sugars (glucose, fructose, ...), acids (acetate, succinate, ...)
    - [CO_2, methane]
  - N: ammonium (NH_4^+), nitrate (NO_3^-), ...
  - mixed C/N: amino acids, NAG
  - phosphate, sulfate, ... [O_2]
- micronutrients: metal, vitamins
- buffer (range, capacity)
- osmolarity

Growth venue:

- batch culture growth

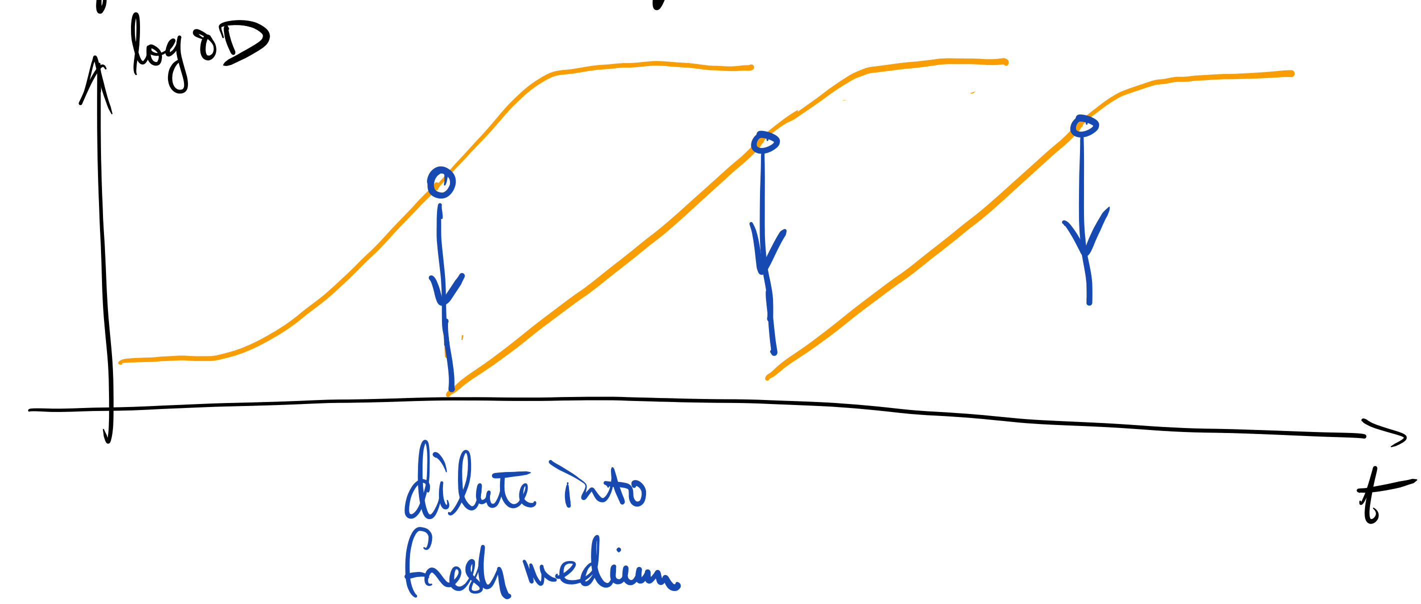


optical density: biomass density
= mass/cell × cell/ml
= ρ

↑ ∝ cell density if cell size const

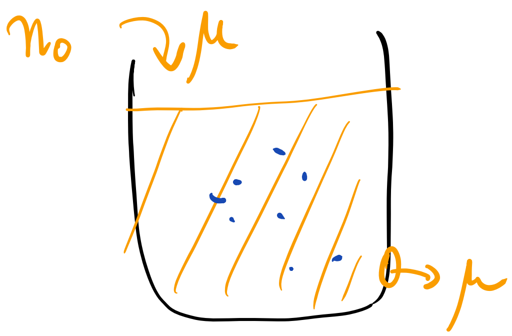
1 ml culture at $OD_{600} = 1$:
0.5 mg drymass
(in 1g of water)

- growth-dilution cycle



$\Rightarrow$ Steady state growth (balanced growth)
if lag and stationary phase avoided.

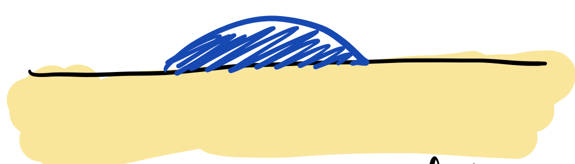
- Continuous culture (chemostat)



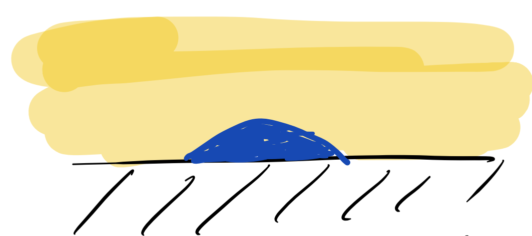
slow relaxation
towards steady state
if close to washout limit.

Above are all planktonic growth

bacteria growth on solid substrate



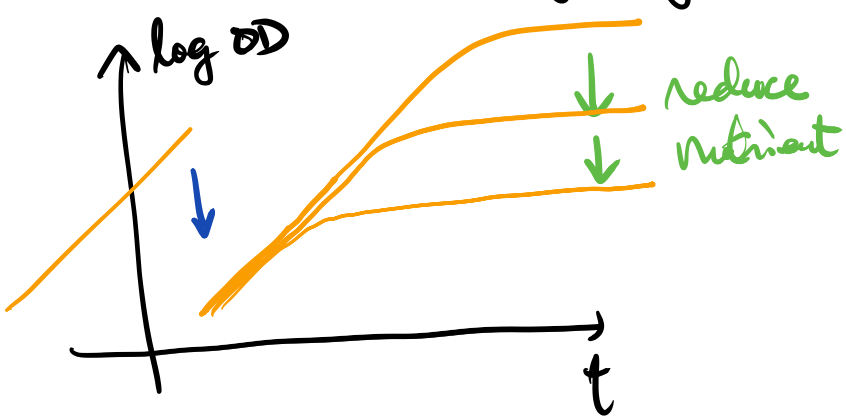
nutrient provided by subs.

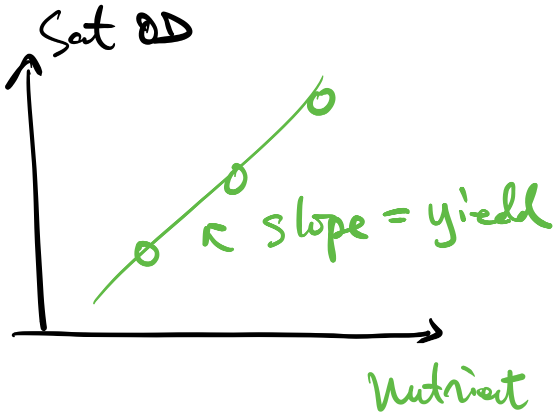


nutrient provided by fluid

batch culture growth

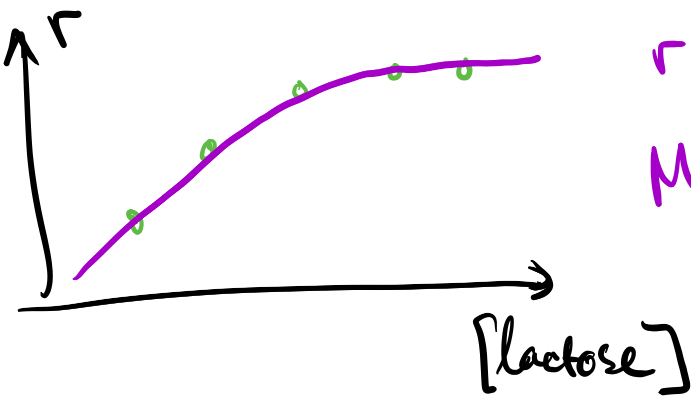
(with enough cycling to enter steady state)





for E. coli, $Y_{glc} \simeq 1\,OD / 5\,mM$ glucose

→ nutrient conc also affect growth rate



$$r = r_0 \frac{n}{n + K_M}$$

Monod growth kinetics (1942)

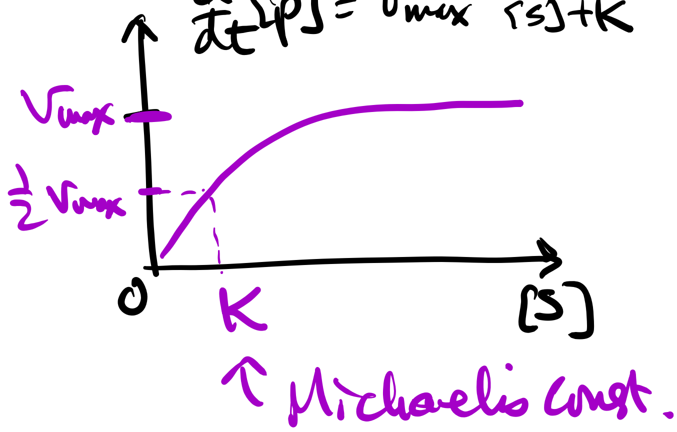
r_0 sat. growth rate

K_M Monod constant.

Same form as Michaelis-Menten enzyme kinetics:

$$E + S \underset{k_{1-}}{\overset{k_{1+}}{\rightleftharpoons}} E\cdot S \xrightarrow{k_2} E + P.$$

$$\frac{d}{dt}[p] = V_{max} \frac{[S]}{[S]+K}$$



$$\frac{d}{dt}[p] = k_2 \cdot [E\cdot S]$$

$$\frac{[E\cdot S]}{[E]_{free} \cdot [S]} = \frac{k_{1+}}{k_{1-}}$$

$$[E]_{tot} = [ES] + [E]_{free} = [ES] + \frac{[ES]}{[S]}\frac{k_{1-}}{k_{1+}}$$

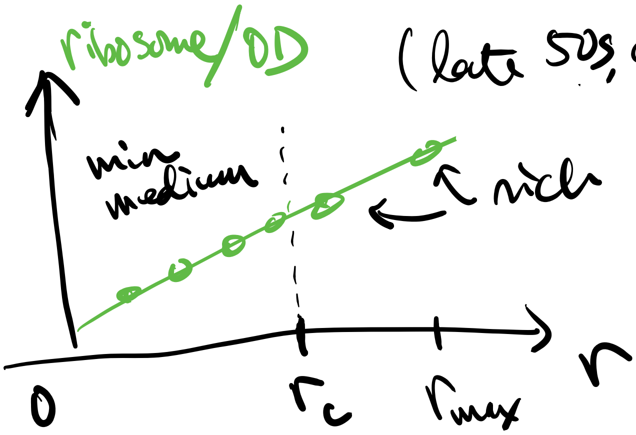
$$[ES] = \frac{[E]_{tot}}{1 + \frac{k_{1-}}{k_{1+}}/[S]}$$

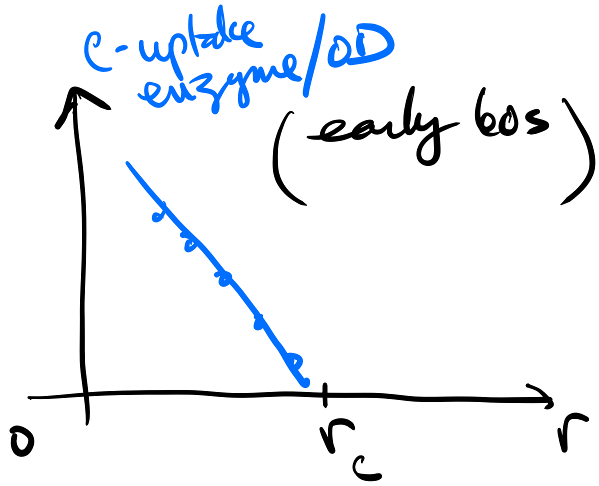
$$\rightarrow \frac{d}{dt}[P] = \underbrace{k_2 [E_{tot}]}_{V_{max}} \cdot \frac{[S]}{[S] + \underbrace{k_{1-}/k_{1+}}_{K}}$$

but what does M/M Kinetics has to do with cell growth ??

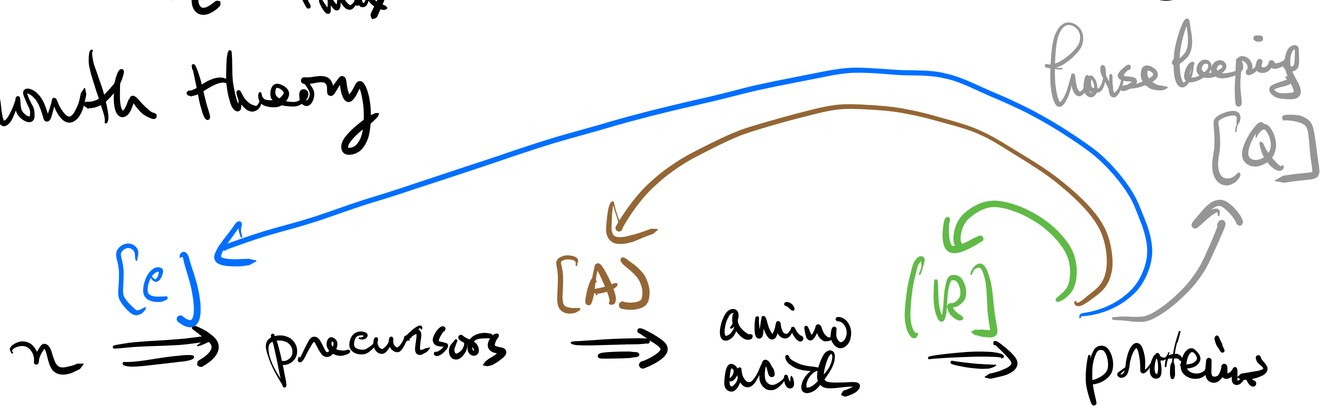
growth limited by nutrient uptake ?
(e.g. E = uptake enzyme?)

b) bacterial growth laws





growth theory



total cell mass $\simeq$ total protein mass $= M$.

total ribosomal mass $= M_R$

total mass of protein X $= M_X$

$\frac{dM}{dt} = k_R M_R$ $\quad k_R$: Specific rate of ribosome activity or translational elongation rate

exponential growth $\frac{dM}{dt} = r \cdot M = k_R M_R(t)$

$\Rightarrow \frac{M_R(t)}{M(t)} = r / k_R$

↑ ribosomal mass fraction

$\propto$ ribosome/OD (rationalization of growth law)

$\propto [R]$ Since M/water is invariant

" total protein conc.

gene regulation:

$\frac{dM_i}{dt} = \chi_i k_R M_R$ $\quad$ fraction of ribosomes synthesizing protein i.

$\longrightarrow \frac{M_i}{M} = \chi_i$ $\quad$ sets protein conc.

What should protein conc be set to?

Carbon uptake flux: $w_c M_c = -\frac{dn_c}{dt} = Y_c^{-1} \frac{dM}{dt}$

↑ Specific uptake rate

$Y_c \frac{dn_c}{dt} + \frac{dM}{dt} = 0$

$\longrightarrow \frac{M_c}{M} = \frac{r}{w_c Y_c} \equiv \frac{r}{k_c}$

Internal flux balance : $k_c M_c = k_A M_A = \frac{dM}{dt}$

$$\rightarrow \frac{M_A}{M} = \frac{r}{k_A}$$

Overall constraint :

$$\frac{M_c}{M} + \frac{M_A}{M} + \frac{M_R}{M} + \frac{M_Q}{M} = 1$$

assume $\frac{M_Q}{M} = \text{const}$ (expt $\frac{M_Q}{M} \approx 50\%$)

$$\frac{r}{k_R} + \frac{r}{k_c} + \frac{r}{k_A} = \phi_{max} = 1 - \frac{M_Q}{M}$$

fraction of proteome available to growth-dependent processes

rich medium : $k_c = \infty$, $k_A = \infty$.

$$r = r_{max} = k_R \phi_{max} \quad (\approx 2/h \text{ for E. coli})$$

min medium with best c-source : $k_c = \infty$.

$$r = r_c = \frac{\phi_{max}}{k_R^{-1} + k_A^{-1}} = k_{RA} \phi_{max}$$

min medium with "poor" c-source small k_c

$$\frac{r}{k_c} + \frac{r}{k_{RA}} = \phi_{max}$$

$$r = \frac{k_{RA}\phi_{max}}{1 + k_{RA}/k_c} = r_c \frac{x}{1+x}$$

$x = \frac{k_c}{k_{RA}}$: carbon "quality" (glucose $x \approx 10$, $r \approx r_c$)

- Relation between C-proteins and GR?

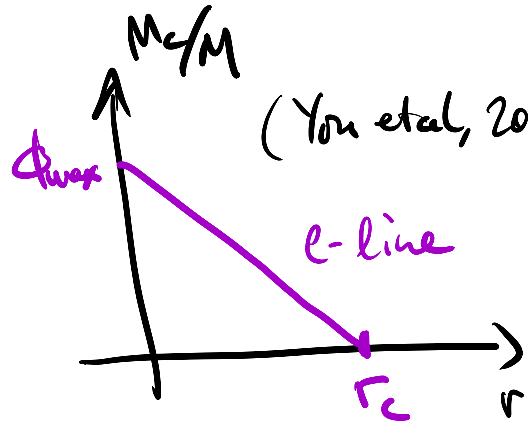
under C-limitation (changing C-sources, i.e., $k_c Y_c$)

$$\frac{M_C}{M} = \phi_{max} - \frac{M_R}{M} - \frac{M_A}{M}$$

$$= \phi_{max} - \frac{r}{k_{RA}}$$

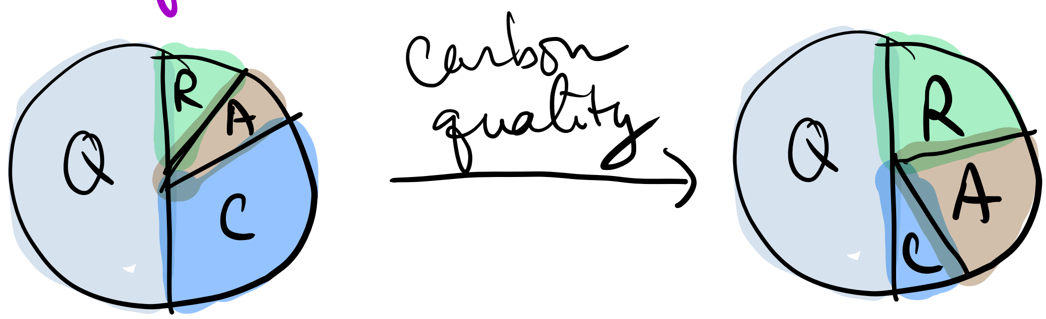
Since $r_c = k_{RA}\phi_{max}$,

$$\frac{M_C}{M} = \phi_{max}\cdot\left(1 - r/r_c\right)$$



→ explains "catabolite repression"

an ubiquitous phenomenon in microbial.



- Conc dependence of GR?

include MM kinetics of uptake protein

$$k_c \to k_c \frac{n}{n+K_c} \quad \text{or} \quad k_c^{-1} \to k_c^{-1}\left(1+\frac{K_c}{n}\right)$$

$$\to r = \frac{r_c}{1+\frac{k_{RA}}{k_c}\cdot\left(1+\frac{K_c}{n}\right)} = \frac{r_c}{1+\frac{k_{RA}}{k_c}+\frac{k_{RA}}{k_c}\cdot\frac{K_c}{n}}$$

$$r = \frac{r_{sat}}{1 + \frac{K_M}{n}}$$ recovers Monod!

$$r_{sat} = r_c \frac{x}{1+x}$$ $x = k_c / k_{RA}$

$$K_M = \frac{K_c}{1+x}$$ reduced due to adjustment in C-protein expression

→ good C-source tends to have small K_M.

→ However, nature of transporter (k_c) also can play important role
(e.g. ABC transporters tend to have small K_c)

c) two substitutable nutrients (C-sources)

Consider nutrient C_1 and C_2

if GR on individual C-source is r_1, r_2.
what is GR on both, r_{12}?

for E.coli two types of utilization are known

- hierarchical: $r_{12} = \max\{r_1, r_2\}$
  requires special regulatory interaction
- simultaneous: $r_{12} = r_1 \oplus r_2$

(but can't be a simple sum since $r_{12} < r_c$)

$$\omega_{c1} M_{c1} = -\dot{n}_1 \qquad M_{c1} = \alpha_1 M_c$$ follow

$$\omega_{c2} M_{c2} = -\dot{n}_2 \qquad M_{c2} = \alpha_2 M_c$$ c-line

find from single c-source

flux matching: $(c_1 + c_2)$

$$k_A M_A = \omega_{c1} Y_1 M_{c1} + \omega_{c2} Y_2 M_{c2}$$

$$k_R M_R = k_A M_A = \frac{dM}{dt} = r_{12} M$$

C_1 alone

$$\omega_{c1} Y_1 M_{c1} \equiv k_{c1} M_{c1} = r_1 M$$

since

$$\frac{M_{c1}}{M} = \alpha_1 \phi_{max} \left(1 - \frac{r_1}{r_c}\right)$$

we have

$$\alpha_1 k_{c1} = \frac{r_1}{\phi_{max}\left(1 - \frac{r_1}{r_c}\right)}$$

similarly,

$$\alpha_2 k_{c2} = \frac{r_2}{\phi_{max}\left(1 - \frac{r_2}{r_c}\right)}$$

for $C_1 + C_2$:

$$\frac{M_{c1}}{M} = \alpha_1 \phi_{max} \left(1 - \frac{r_{12}}{r_c}\right)$$

$$\frac{M_{c2}}{M} = \alpha_2 \phi_{max} \left(1 - \frac{r_{12}}{r_c}\right)$$

So, $k_{c1} M_{c1} + k_{c2} M_{c2} = r_{12} M$ becomes

$$\left(k_{c1}\alpha_1 + k_{c2}\alpha_2\right) \cdot \phi_{max}\left(1 - \frac{r_{12}}{r_c}\right) = r_{12}$$

or

$$\frac{r_1}{1 - \frac{r_1}{r_c}} + \frac{r_2}{1 - \frac{r_2}{r_c}} = \frac{r_{12}}{1 - \frac{r_{12}}{r_c}}$$

$$\rightarrow \quad r_{12} = \frac{r_1 + r_2 - 2 r_1 r_2 / r_c}{1 - r_1 r_2 / r_c^2} \qquad \left(\text{Hermsen et al } 2014\right)$$

$$\simeq \begin{cases} r_1 + r_2 & \text{if } r_1, r_2 \ll r_c \\ r_c & \text{if } r_1, r_2 \simeq r_c \end{cases}$$

if the conc n_1 and n_2 are low,

then $\quad r_{12} \simeq r_1 \dfrac{n_1}{K_1} + r_2 \dfrac{n_2}{K_2}$

d) two essential nutrients (e.g. C and N)

$\rightarrow$ growth rate drops if either is low.

guess: $r(n_c, n_N) = r_{sat} \dfrac{n_c}{n_c + K_{M,C}} \dfrac{n_N}{n_N + K_{M,N}}$ (wrong!)

$$\omega_c(n_c) M_c = -\frac{dn_c}{dt} = Y_c^{-1} \frac{dM}{dt}$$

$$\omega_N(n_N) M_N = -\frac{dn_N}{dt} = Y_N^{-1} \frac{dM}{dt}$$

Internal flux balance $\quad \overset{N}{\longrightarrow} \underset{e}{\longrightarrow} \longrightarrow AA.$

$$\underbrace{\omega_c Y_c}_{\equiv k_c} M_c = \underbrace{\omega_N Y_N}_{\equiv k_N} M_N = k_A M_A = k_R M_R = r M$$

$$\frac{M_c}{M} = \frac{r}{k_c}, \quad \frac{M_N}{M} = \frac{r}{k_N}, \quad \frac{M_A}{M} = \frac{r}{k_A}, \quad \frac{M_R}{M} = \frac{r}{k_R}$$

$$\frac{M_c}{M} + \frac{M_N}{M} + \frac{M_A}{M} + \frac{M_R}{M} = 1 - \frac{M_Q}{M} = \phi_{max}$$

$$\frac{r}{k_C} + \frac{r}{k_N} + \frac{r}{k_{RA}} = \phi_{max}$$

$$r = \frac{\phi_{max}}{k_{RA}^{-1} + k_C^{-1} + k_N^{-1}} = \frac{r_c}{1 + \frac{k_{RA}}{k_C} + \frac{k_{RA}}{k_N}}$$

include MM dependence on uptake

$$k_C^{-1}(n_C) = k_C^{-1}\left(1 + \frac{K_C}{n_C}\right)$$

$$k_N^{-1}(n_N) = k_N^{-1}\left(1 + \frac{K_N}{n_N}\right)$$

$$r = \frac{r_c}{1 + \frac{k_{RA}}{k_C}\left(1 + \frac{K_C}{n_C}\right) + \frac{k_{RA}}{k_N}\left(1 + \frac{K_N}{n_N}\right)}$$

$$= \frac{r_{sat}}{1 + \frac{K_{MC}}{n_C} + \frac{K_{MN}}{n_N}} \neq \frac{r_{sat}}{\left(1 + \frac{K_{MC}}{n_C}\right)\cdot\left(1 + \frac{K_{MN}}{n_N}\right)}$$

For $n_C \ll K_{MC}$, $n_N \ll K_{MN}$

$$r = \frac{r_{sat}}{\frac{K_{MC}}{n_C} + \frac{K_{MN}}{n_N}} \neq \frac{r_{sat}}{\frac{K_{MC}}{n_C} \cdot \frac{K_{MN}}{n_N}}$$