$$r = \frac{r_0}{1 + \frac{K_M}{n}}$$ recovers Monod!

$$r_0 = r_c \frac{x}{1+x}$$ $x = k_c / k_{RA}$

$$K_M = \frac{K_c}{1+x}$$ 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 $r_{12} < r_c$)

flux matching : $r_{12} M = \dot{M} = Y_1 \dot{n}_{c_1} + Y_2 \dot{n}_{c_2}$
$(C_1 + C_2)$

$$\dot{n}_{c_1} = w_{c_1} M_{c_1} \,, \quad \dot{n}_{c2} = w_{c_2} M_{c_2}$$

$$\Rightarrow r_{12} M = \underbrace{w_{c_1} Y_1}_{k_{c_1}} M_{c_1} + \underbrace{w_{c2} Y_2}_{k_{c2}} M_{c2}$$

Co-regulation of C-uptake : $M_{c_1} = \eta_1 M_c \,, \quad M_{c2} = \eta_2 M_c$ follow C-line

$$\to k_{c_1} \frac{M_{c_1}}{M} + k_{c_2} \frac{M_{c2}}{M} = (k_{c_1}\eta_1 + k_{c_2}\eta_2)\frac{M_c}{M} = r_{12}$$

$$\text{or } k_{c_1}\eta_1 + k_{c2}\eta_2 = \frac{r_{12}}{\phi_{max}\left(1 - \frac{r_{12}}{r_c}\right)}$$

C_1 alone $(GR = r_1)$: $\eta_1 k_{c_1} = \dfrac{r_1}{\phi_{max}\left(1 - \frac{r_1}{r_c}\right)}$

C_2 alone $(GR = r_2)$: $\eta_2 k_{c2} = \dfrac{r_2}{\phi_{max}\left(1 - \frac{r_2}{r_c}\right)}$

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

$$\text{or } r_{12} = \frac{r_1 + r_2 - 2 r_1 r_2 / r_c}{1 - r_1 r_2 / r_c^2}$$

(Hermsen et al 2014)

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

$\Rightarrow$ if conc n_1, n_2 low, then $r_{12} \simeq r_1 \dfrac{n_1}{K_1} + r_2 \dfrac{n_2}{K_2}$

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

→ growth rate drops if <u>either</u> 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

$$N,\ C \longrightarrow AA.$$

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

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

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

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

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

include MM dependence on uptake

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

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

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

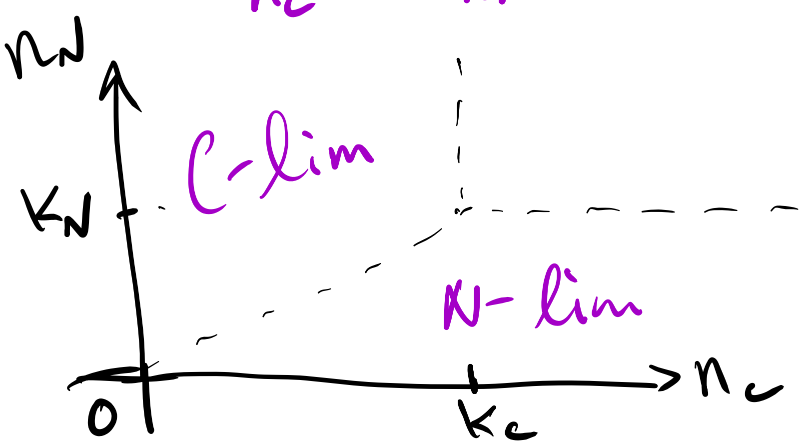
$$= \frac{r_0}{1+\frac{K_{MC}}{n_C}+\frac{K_{MN}}{n_N}} \neq \frac{r_0}{\left(1+\frac{K_{MC}}{n_C}\right)\cdot\left(1+\frac{K_{MN}}{n_N}\right)}$$

$$r_0 = r_C \Big/ \left(1+\frac{k_{RA}}{k_C}+\frac{k_{RA}}{k_N}\right)$$

$$K_{MC} = K_C \cdot \frac{k_{RA}}{k_C} \Big/ \left(1+\frac{k_{RA}}{k_C}+\frac{k_{RA}}{k_N}\right)$$

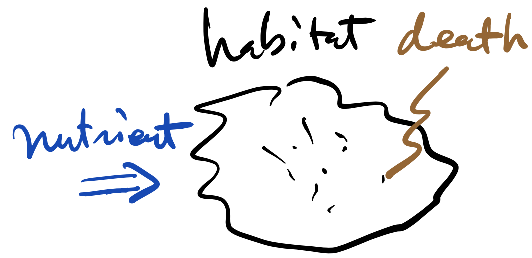
For $n_C \ll K_{MC}$, $n_N \ll K_{MN}$

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

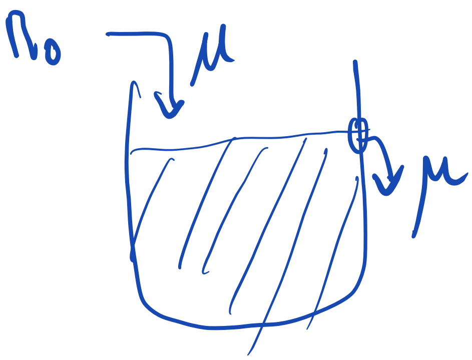


2. Continuous culture of single species

Common scenario: nutrient influx j_0, death at rate δ



Mimicked by a chemostat:



- nutrient of conc n_0 dripping in at rate μ $(j_0 = \mu \cdot n_0)$
- medium (incl cells) removed at rate μ $(\delta = \mu)$

$$\begin{cases} \dfrac{d\rho}{dt} = r(n)\rho - \mu\rho \\ \dfrac{dn}{dt} = n_0\mu - n\mu - r(n)\rho/Y \end{cases}$$

- Monod growth law: $r(n) = r_0 \dfrac{n}{n+K}$
- Yield: $Y = \dfrac{\delta\rho}{\delta n}$

a) Steady state: $n(t) \to n^* \leq n_0$ ← nutrient in medium

$\rho(t) \to \rho^* \geq 0$ ← density of culture

Constraint (indept of $r(n)$): $\rho^* = \underbrace{(n_0 - n^*)}_{\text{consumed nutrient}} Y$ (mass conservation)

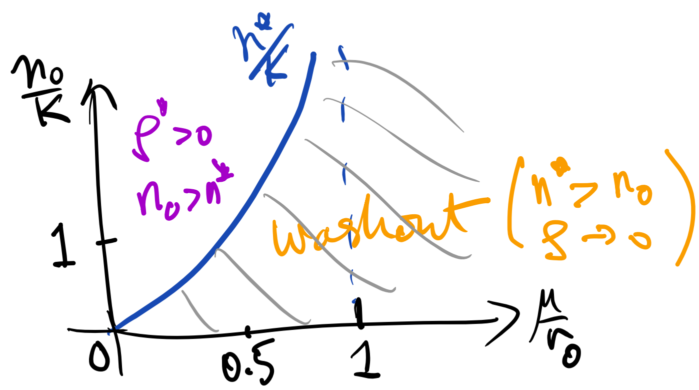
Check: $\dfrac{d\rho}{dt} + Y \cdot \dfrac{dn}{dt} = \mu \cdot [(n_0 - n(t))Y - \rho(t)] \xrightarrow{\frac{d}{dt}=0} \mu(n_0 - n^*)Y - \rho^*$

fixed pt: $r(n^*) = \mu$

$\to \dfrac{\mu}{r_0} = \dfrac{n^*}{n^*+K} \to \dfrac{n^*}{K} = \dfrac{\mu}{r_0 - \mu}$

further, $\rho^* > 0 \to n_0 > n^*$

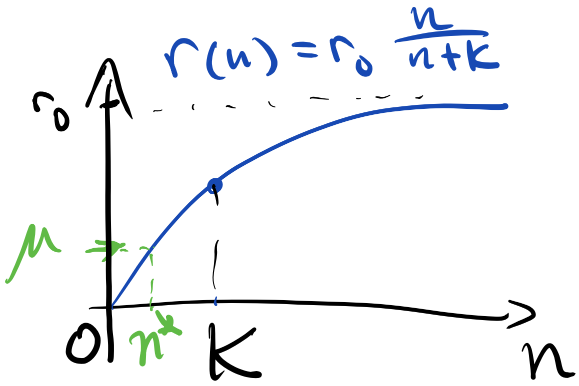
$$\Rightarrow \frac{n_0}{K} > \frac{\mu}{r_0 - \mu} > 0$$



Note: $j_0 = \mu n_0$: environmental

r_0, K, μ : physiological

general rule: chemostat culture "washes out" if μ too large or n_0 too small.



Common: $\mu \ll r_0$

$\rightarrow n^* \ll K$.

- Can linearize Monod:

$$r(n) \simeq \frac{r_0 n}{K} \equiv \nu n \quad \xleftarrow{} r_0/K$$

(Will work with $\mu \ll r_0$ throughout, and use $r(n) = \nu n$)

Criterion for stable chemostat culture becomes

$$\frac{n_0}{K} > \frac{\mu}{r_0 - \mu} \simeq \frac{\mu}{r_0} \rightarrow \mu < r_0 n_0 / K = \nu n_0$$

$\rightarrow$ one dimensionless parameter $\eta = \frac{\mu}{\nu n_0}$

$\rightarrow$ stability of chemostat requires $\eta < 1$

[Note that $\frac{n^*}{K} = \frac{\mu}{r_0 - \mu} \overset{\mu \ll r_0}{\simeq} \frac{\mu}{r_0} \rightarrow \frac{n^*}{n_0} \simeq \frac{\mu K}{r_0 n_0} = \eta$

from mass conservation $\rho^* = (n_0 - n^*) Y$

also get $\frac{\rho^*}{\rho_0} = 1 - \eta$ where $\rho_0 \equiv n_0 Y$ is max density

$\rightarrow$ can est η (hence K) from $\frac{n^*}{n_0}$ or $\frac{\rho^*}{\rho_0}$]