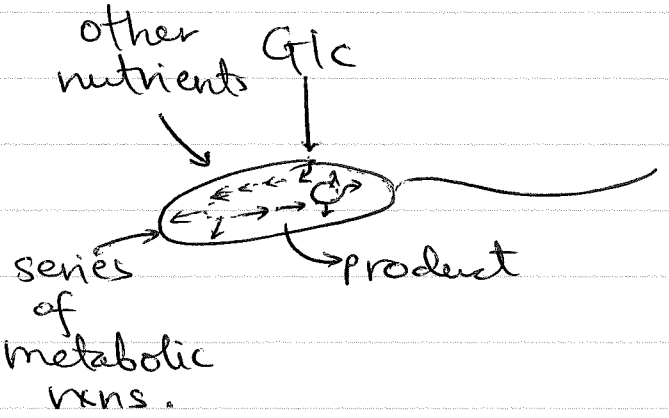
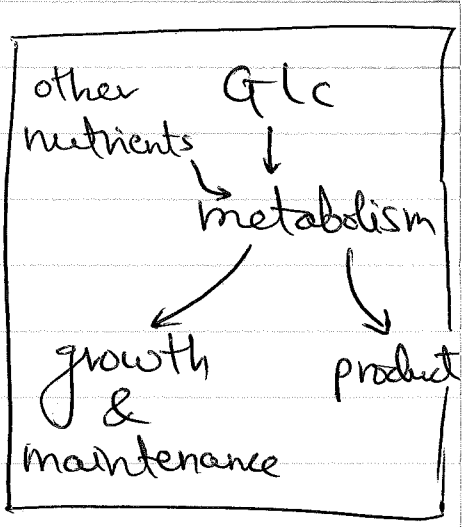
Fermentation & bioreactors



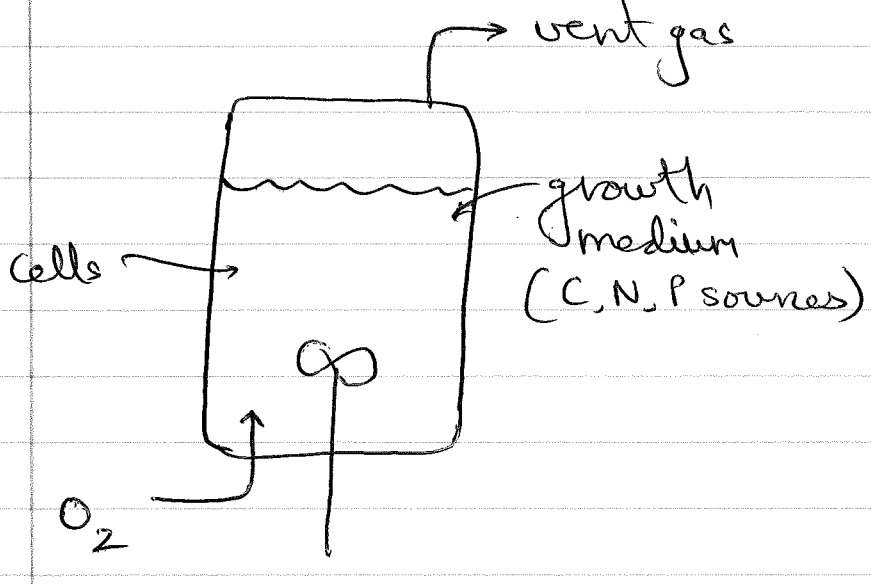


Therefore, the general reaction for cell growth is:

$$\text{cells} + \text{C source} + \text{N source} + \text{P source} + \text{O source} \rightarrow \text{more cells} + \text{product} + CO_2 + H_2O$$

$\Rightarrow$ Common nutrients: C ≡ glucose, glycerol
N ≡ ammonium salts
P ≡ phosphate salts
O ≡ O_2 gas

$\Rightarrow$ oxygen might be optional → aerobic / anaerobic



* Fermenters can be operated either in batch mode or as CSTRs

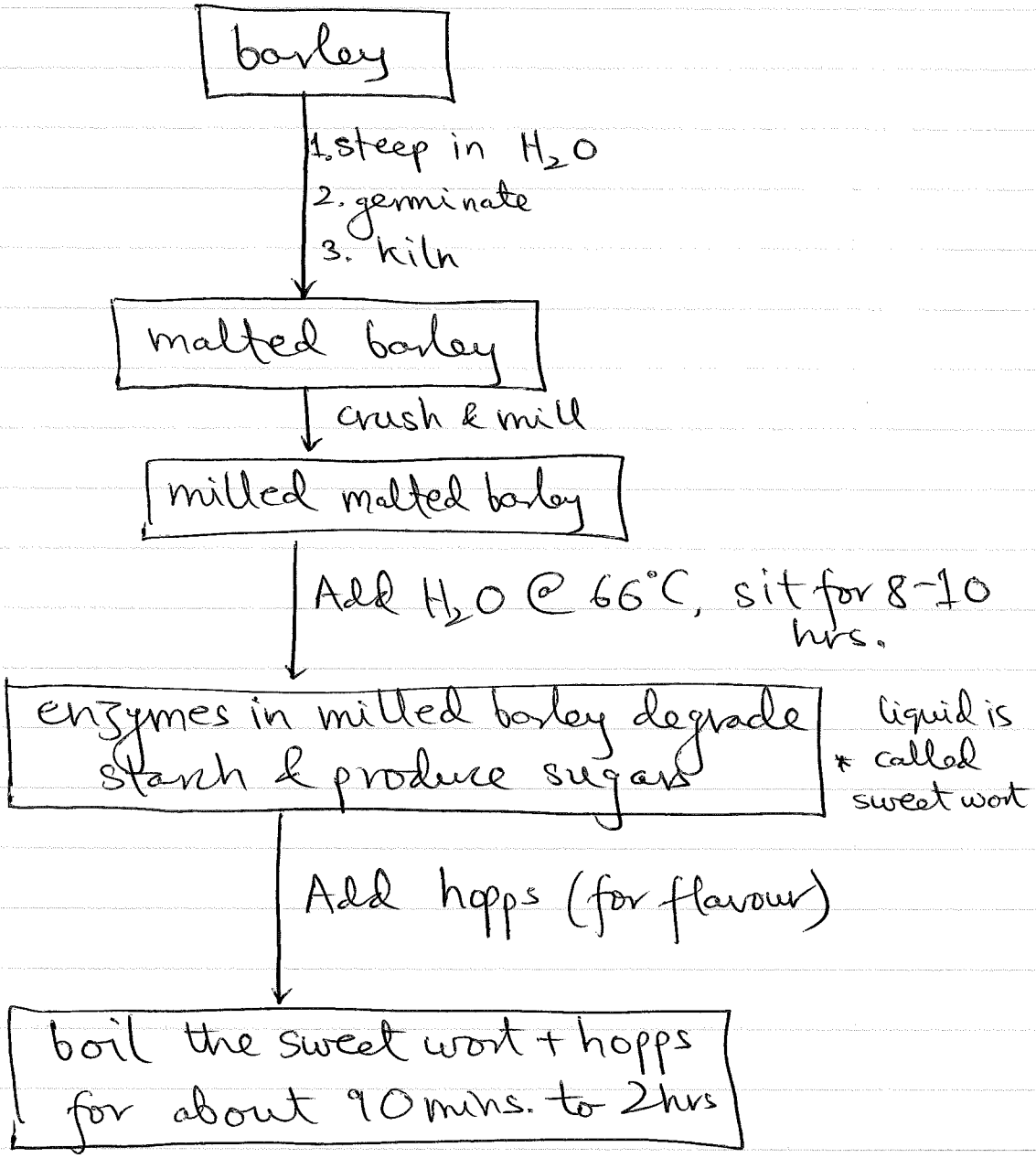
* The mode of operation is dependent on the nature of the product & 3 terms
  - ↳ yield (g/g)
  - → productivity (g/L.s)
  - → titre (g/L)

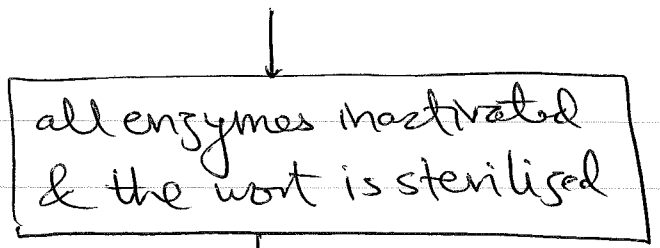
nature of product
- → growth associated (primary metabolite)
- → non-growth associated (secondary metabolites) like antibiotics

Most common example of a bioprocess

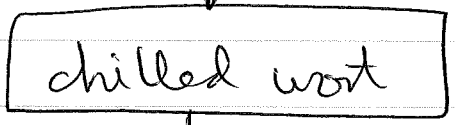
* brewing beer

Let's relate the process of brewing beer to the basics of fermentation.





pass through heat exchanger



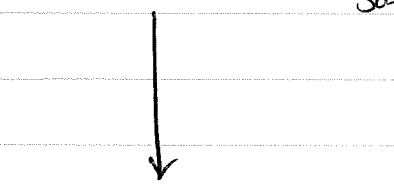
* the chilled wort is partially aerated ∴ brewing is really micro-aerobic

Add yeast →

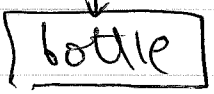
3-7 days

{ 20-23°C ales | 7-12°C lagers }

common strains: pure cultures of
Saccharomyces cerevisiae
Saccharomyces carlsbergensis



* for making the beer fizzy



* production of sweet wort ≡ C, N & P source

yeast — wort

cells + C source + N source + P source

↓ temperature, pH

more cells + CO_2 + H_2O + C_2H_5OH

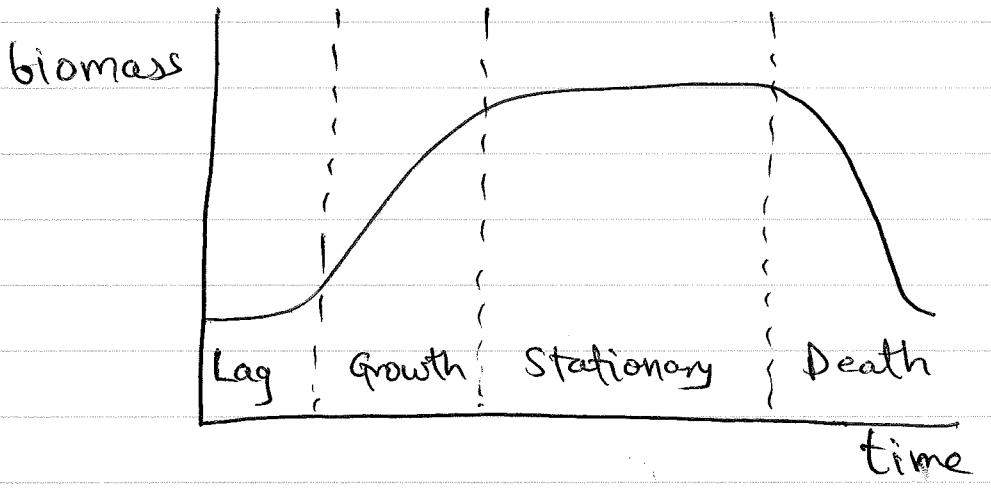
filtered & removed — fizz — beverage

* since anaerobic metabolism is not conducive to rapid growth, cell mass is not very high
  → brewing is typically batch

* if cell mass is a problem, continuous operation might have to be considered

Calculations for design of a fermentation process:

Cell growth



Let concentration of cells = C_c

$\frac{dC_c}{dt} = 0$ in lag & stationary phases

* you don't want to operate in the death phase (so let's not consider this) for now .... we'll look @ this in mass balances

Let's look at the growth phase.

$$r_G = \frac{dC_c}{dt} = \mu C_c$$

{in bioprocess eng: units are mass/volume}

..... μ has units of s^{-1}

r_G : rate of growth

μ : specific growth rate

And,

$$\mu = \frac{\mu_{max} C_s}{K_s + C_s}$$ ..... Monod equation

μ_{max} ≡ maximum specific growth rate

C_s ≡ concentration of nutrient (e.g. glucose)

↑ usually the limiting nutrient

The Monod equation is very similar to the Michaelis-Menten equation.

* behaviour w.r.t. temperature, pH similar to enzymes
* Jacques Monod ≡ also a Nobel Laureate

In addition to the Monod equation, there are 2 other equations that are also used from time to time

* Moser equation

$$\mu = \mu_{max}\left[1 - \exp\left(\frac{-C_s}{K}\right)\right]$$

* Tessier equation

$$\mu = \frac{\mu_{max}}{1 + K C_s^{-\lambda}}$$

In these equations, K & λ are empirical constants that are obtained through data fitting

What happens to the cells during brewing?

* As cells produce ethanol, the ethanol begins to become toxic to the cells

* This is called product inhibition

If <u>product inhibition</u> is observed,

$$\mu = \left\{1 - \frac{C_p}{C_p^*}\right\}^n \frac{\mu_{max} C_s}{K_s + C_s}$$

Here, C_p^* ≡ concentration of product where all metabolism ceases

Yields, productivity & titer

Titer ≡ $\frac{g}{L}$ i.e. C_c, C_p, C_s etc.

$$\text{productivity} \equiv \frac{\text{titers observed}}{\text{time of fermentation}}$$

yields ⟹ most important, especially for calculations

$$Y_{A/B} \equiv \frac{\text{mass of A}}{\text{mass of B}}$$

↖ yield of A w.r.t. B

For cells, $Y_{c/s} \equiv \frac{\text{mass of cells formed}}{\text{mass of substrate consumed}}$

Also, $\boxed{Y_{c/s} = \frac{1}{Y_{s/c}}}$

For product, $Y_{p/c} \equiv \frac{\text{mass of product formed}}{\text{mass of cells formed}}$

How does this matter in calculations?

product formation → growth-associated (growth phase)

product formation → non-growth associated (stationary phase)

Growth associated:

$$r_p = Y_{p/c} r_G$$

$$r_G = \mu C_c = \frac{\mu_{max} C_s C_c}{K_s + C_s}$$

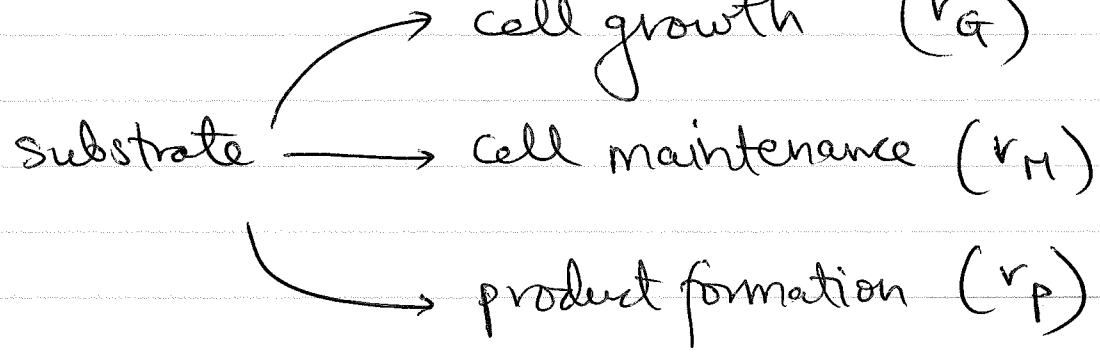
$$\therefore r_p = \frac{Y_{p/c}\, \mu_{max} C_s C_c}{K_s + C_s}$$

For non-growth associated product formation:

$$r_p = Y_{p/s} \cdot \left(-r_s^{st}\right)$$

all substrate is directed to product formation during stationary phase

Let's look at substrate utilization:



$$-r_s = Y'_{s/c} r_G + Y'_{s/p} r_p + Y'_{s/M} r_M$$

$Y'_{s/c}$: exclusive yield

$Y'_{s/M}$: general form

What do we mean by exclusive yield?

$$Y_{S/C} \equiv \frac{\text{mass of substrate consumed}}{\text{mass of cells formed}}$$

$$\text{But } \left\{\begin{matrix}\text{mass of}\\ \text{substrate}\\ \text{consumed}\end{matrix}\right\} = \left\{\begin{matrix}\text{mass of}\\ \text{substrate}\\ \text{consumed}\\ \text{to form}\\ \text{new cells}\end{matrix}\right\} + \left\{\begin{matrix}\text{mass of}\\ \text{substrate}\\ \text{consumed}\\ \text{to form}\\ \text{products}\end{matrix}\right\} + \ldots$$

$$-r_S \equiv \frac{\text{mass of substrate consumed}}{\text{time}}$$

$$r_G = \frac{\text{mass of cells formed}}{\text{time}}$$

$$\therefore \frac{\left\{\begin{matrix}\text{mass of}\\ \text{substrate}\\ \text{consumed}\end{matrix}\right\}}{\text{time}} = \left\{\begin{matrix}\text{mass of}\\ \text{substrate}\\ \text{consumed}\\ \text{to form}\\ \text{new cells}\end{matrix}\right\} \times \frac{\left\{\begin{matrix}\text{mass of cells}\\ \text{formed}\end{matrix}\right\}}{\text{time}} + \ldots\ldots$$

$$\left\{\begin{matrix}\text{mass of cells}\\ \text{formed}\end{matrix}\right\}$$

i.e. $$-r_S = \left(Y'_{S/C} * r_G\right) + \left(Y'_{S/P}\, r_P\right) + \ldots\ldots$$

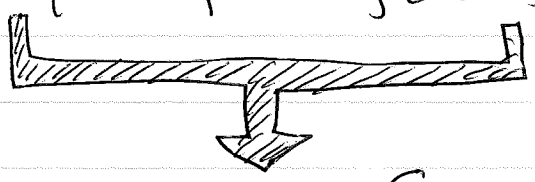
$$\therefore \quad -r_S = Y'_{S/C} r_G + Y'_{S/P} r_P + Y'_{S/M} r_M$$

what is rate of maintenance?

$$Y'_{S/M} r_M = \frac{\left\{\begin{matrix}\text{mass of substrate} \\ \text{consumed for} \\ \text{maintenance}\end{matrix}\right\}}{\{\text{mass cells formed}\}} \times \frac{\{\text{mass of cells formed}\}}{\{\text{time}\}}$$

$$\therefore \quad Y'_{S/M} r_M = \frac{\left\{\begin{matrix}\text{mass of substrate} \\ \text{consumed for} \\ \text{maintenance}\end{matrix}\right\}}{\{\text{mass of cells formed}\}\{\text{time}\}} \times \left\{\begin{matrix}\text{mass of} \\ \text{cells} \\ \text{formed}\end{matrix}\right\}$$

$$\text{i.e.} \quad Y'_{S/M} r_M = \frac{\left\{\begin{matrix}\text{mass of substrate} \\ \text{consumed for} \\ \text{maintenance}\end{matrix}\right\}}{\{\text{mass of cells}\}\{\text{time}\}} \times \left\{\begin{matrix}\text{mass of} \\ \text{cells}\end{matrix}\right\}$$



$m \cdot C_c$

$$-r_S = Y'_{S/C} r_G + Y'_{S/P} r_P + m C_C$$

Consider a case where a product is non-growth associated & is formed from a secondary nutrient.

Recollect, $r_P = Y_{P/SN}\left(-r_{SN}^{st}\right)$

since the 2° nutrient is only consumed in the stationary phase, we can rewrite this as $-r_{SN}$ only.

$$\therefore \quad r_P = Y_{P/SN} \cdot (-r_{SN}) \longrightarrow (1)$$

Let's assume one can write the following mechanism :-

$$S_N + E \rightleftharpoons ES_N \rightarrow E + P$$

$$\therefore \quad r_P = \frac{k_3 [E]_o [S_N]}{K_M + [S_N]}$$

$$\therefore \quad r_p = \frac{k_3 C_{E_0} C_{SN}}{K_M + C_{SN}}$$

But $k_3 C_{E_0}$ corresponds to the cell concentration

$$\therefore \quad k_3 C_{E_0} \approx k_p C_c$$ .... k_p is defined as the specific rate constant w.r.t. product ($m^3/g.s$)

K_M can be equated to Monods eqn.

$$K_M = \frac{[E][S_N]}{[ES_N]} \approx K_{SN}$$

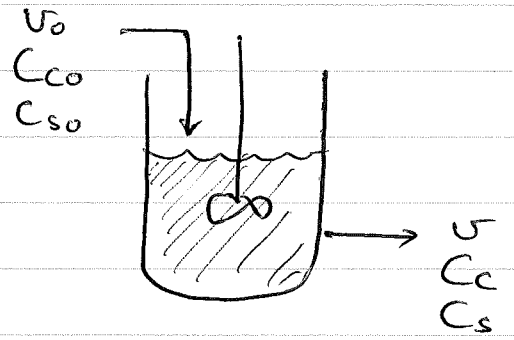
$[ES_N]$: can be related to cell mass

K_{SN}: Monod

$$\therefore \quad r_p = \frac{k_p C_c C_{SN}}{K_{SN} + C_{SN}} \longrightarrow (2)$$

$$\therefore \quad -r_{SN} = \frac{1}{Y_{P/SN}} \left(\frac{k_p C_c C_{SN}}{K_{SN} + C_{SN}} \right)$$

Mass balances in bioreactors



$$\therefore \quad \frac{d(VC_c)}{dt} = v_0 C_{c0} - v C_c + (r_g - r_d) V$$

Like the growth rate, the deathrate can be related to the existing cell population as well as toxins that might accumulate

$$\therefore \quad r_d = (k_d + k_t C_t) C_c$$

why this form?

death in a resource limited environment $\propto$ no. of individuals

$\propto$ direct interaction between cells & toxin

Similarly, for substrate:

$$\frac{d(VC_s)}{dt} = v_0 C_{s0} - v C_s + r_s V$$

↳ Remember our sign conventions

Also, $\frac{d(VC_p)}{dt} = v_0 C_{p0} - v C_p + r_p V$

Let's look at a batch reactor:

No flows $\Rightarrow v = v_0 = 0$

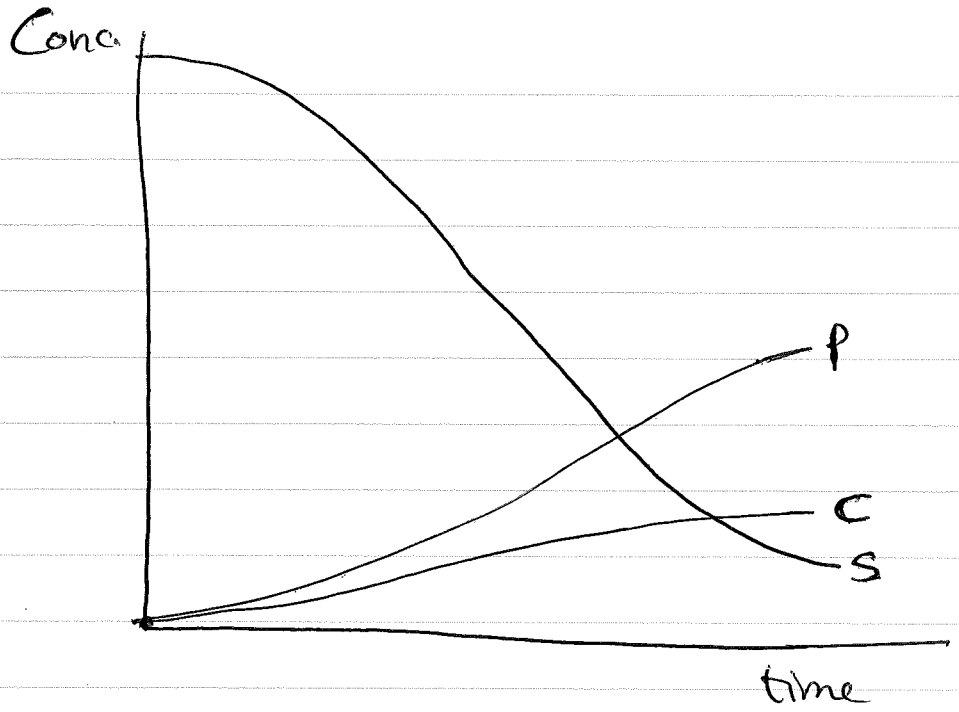
$\therefore$ cells: $\frac{d(VC_c)}{dt} = (r_G - r_D) V$

substrate: $\frac{d(VC_s)}{dt} = r_s V$

product: $\frac{d(VC_p)}{dt} = r_p V$

If volumes are constant,

$$\frac{dC_c}{dt} = (r_G - r_D), \quad \frac{dC_s}{dt} = r_s \quad \& \quad \frac{dC_p}{dt} = r_p$$



* One can introduce all sorts of complexity in this system

* Always state your assumptions very clearly

Chemostats ≡ CST Bioreactors

$$\tau = \frac{V}{v_0} \quad \text{---- residence time (seconds)}$$

$$D = \frac{v_0}{V} = \frac{1}{\tau} \quad \text{---- dilution rate } (sec^{-1})$$

↑ bioprocess parlance

In a chemostat, the cell mass inside the reactor is constant. Fresh media enters, product continuously produced.

$$\frac{d(C_c V)}{dt} = v_0 C_{c_0} - v C_c + (r_G - r_D) V$$

($v_0 C_{c_0}$: typically zero)

$$\therefore \quad -v C_c = -(r_G - r_D) V$$

$$\therefore \quad \frac{v}{V} C_c = r_G - r_D \qquad \text{---- } v = v_0 \text{ Vols. const.}$$

$$\therefore \quad D C_c = r_G - r_D$$

Similarly, for substrate:

$$D[C_{s_0} - C_s] = (-r_s)$$

Often, it is a very good assumption to ignore r_D → the cells are so well nourished, why would they die?

$$\therefore \quad D C_c = r_G = \mu C_c$$

∴ For an ideal chemostat,

$$\boxed{D = \mu}$$

For substrate,

$$D[C_{s0} - C_s] = -r_s$$

neglect cell maintenance
(for same reason we neglect r_D)

$$\therefore D[C_{s0} - C_s] = Y_{s/c}\,\mu\,C_c$$

$$\therefore C_c = \frac{1}{Y_{s/c}}[C_{s0} - C_s] \quad \text{.... since } \mu = D$$

$$\boxed{\therefore C_c = Y_{c/s}[C_{s0} - C_s]} \longrightarrow (1)$$

Also, $D = \mu = \dfrac{\mu_{max} C_s}{K_s + C_s}$

$$\therefore D K_s + D C_s = \mu_{max} C_s$$

$$\therefore \boxed{C_s = \frac{D K_s}{\mu_{max} - D}} \longrightarrow (2)$$

Wash out

If the dilution rate exceeds the specific growth rate, we will flush out the chemostat.

Mass balance:

$$\frac{d(VC_c)}{dt} = \underbrace{vC_{c0}}_{\Downarrow = 0} - vC_c + r_G V$$

$r_G V$ → neglect death & cell maintenance

$$\therefore \frac{d(VC_c)}{dt} = -vC_c + \mu C_c V$$

∴ For a constant volume case,

$$\frac{dC_c}{dt} = -\frac{vC_c}{V} + \mu C_c$$

$$\therefore \frac{dC_c}{dt} = (\mu - D)C_c$$

$\Longrightarrow$ when $D > \mu$, $\frac{dC_c}{dt}$ is negative

But recollect,

$$C_c = Y_{c/s}\left[C_{so} - C_s\right] \longrightarrow (1)$$

$$\& \; C_s = \frac{D K_s}{\mu_{max} - D} \longrightarrow (2)$$

∴ Wash out ≡ flow rate @ which C_c drops instantaneously to zero

Combining (1) & (2) above,

$$C_c = Y_{c/s}\left[C_{so} - \frac{D K_s}{\mu_{max} - D}\right]$$

set $C_c = 0$

$$\therefore \quad C_{so} = \frac{D K_s}{\mu_{max} - D}$$

$$\therefore \quad \mu_{max} - D = \frac{D K_s}{C_{so}}$$

$$\therefore D\left[1 + \frac{K_s}{C_{s0}}\right] = \mu_{max}$$

$$\therefore D = \frac{\mu_{max} C_{s0}}{C_{s0} + K_s} \Longrightarrow \text{wash out flow rate}$$

What is the flowrate that maximises the mass of cells produced per volume of the reactor? → productivity

$$\text{mass of cells} = V C_c$$

$$\frac{\text{mass of cells}}{\text{volume}} = \frac{V C_c}{V} = D C_c$$

$$\therefore \text{maxima of } \frac{\text{mass of cells}}{\text{volume}} \Rightarrow \frac{d(DC_c)}{dD} = 0$$

From before,

$$C_c = Y_{c/s}\left[C_{s0} - C_s\right]$$

$$C_s = \frac{D K_s}{\mu_{max} - D}$$

$$\therefore \quad C_c = Y_{c/s}\left[C_{so} - \frac{DK_s}{\mu_{max} - D}\right]$$

$$\therefore \quad DC_c = DY_{c/s}\left[C_{so} - \frac{DK_s}{\mu_{max} - D}\right]$$

$$\therefore \quad \frac{d(DC_c)}{dD} = \frac{d}{dD}\left\{DY_{c/s}C_{so} - \frac{D^2 Y_{c/s}K_s}{\mu_{max} - D}\right\}$$

$$\therefore \quad \frac{d(DC_c)}{dD} = Y_{c/s}C_{so} - \left\{\frac{(\mu_{max} - D)(2DY_{c/s}K_s) + (D^2 Y_{c/s}K_s)}{(\mu_{max} - D)^2}\right\}$$

$$\therefore \quad \frac{d(DC_c)}{dD} = Y_{c/s}C_{so} - \left\{\frac{2DY_{c/s}K_s}{\mu_{max} - D} + \frac{D^2 Y_{c/s}K_s}{(\mu_{max} - D)^2}\right\}$$

Set = 0 for $D_{max\,prod}$.

$$\therefore \quad Y_{c/s}C_{so} = \frac{2D_{max\,prod}Y_{c/s}K_s}{\mu_{max} - D_{max\,prod}} + \frac{D_{max\,prod}^2 Y_{c/s}K_s}{(\mu_{max} - D_{max\,prod})^2}$$

Let's call $D_{max\,prod} \equiv D_{mp}$ or $\underline{\underline{D}}$ for convenience

$$\therefore Y_{x/s} C_{s0} = \frac{2D\, Y_{x/s} K_s (l_{max} - D) + D^2 Y_{x/s} K_s}{l_{max}^2 + D^2 - 2 l_{max} D}$$

$$\therefore l_{max}^2 C_{s0} + C_{s0} D^2 - 2 l_{max} C_{s0} D = 2DK_s l_{max} - 2D^2 K_s + D^2 K_s$$

$$\therefore l_{max}^2 C_{s0} + C_{s0} D^2 - 2 l_{max} C_{s0} D = 2DK_s l_{max} - D^2 K_s$$

$$\therefore D^2 (C_{s0} + K_s) - 2D (l_{max} C_{s0} + l_{max} K_s) + l_{max}^2 C_{s0} = 0$$

$$\therefore D = \frac{2(l_{max} C_{s0} + l_{max} K_s) \pm \sqrt{b^2 - 4ac}}{2(C_{s0} + K_s)}$$

(quadratic roots)

$$b^2 - 4ac = [2 l_{max} (C_{s0} + K_s)]^2 - 4 l_{max}^2 C_{s0} (C_{s0} + K_s)$$

$$= 4 l_{max}^2 (C_{s0} + K_s)^2 - 4 l_{max}^2 C_{s0} (C_{s0} + K_s)$$

$$= (C_{s0} + K_s) [4 l_{max}^2 (C_{s0} + K_s) - 4 l_{max}^2 C_{s0}]$$

$$= (C_{s0} + K_s) 4 l_{max}^2 K_s$$

$$\therefore \sqrt{b^2-4ac} = \left(C_{s0}+K_s\right)^{1/2} 2\,\ell_{max}\left(K_s\right)^{1/2}$$

$$\therefore D = \frac{2\ell_{max}\left(C_{s0}+K_s\right) \pm 2\ell_{max}\sqrt{K_s\left(C_{s0}+K_s\right)}}{2\left(C_{s0}+K_s\right)}$$

$$\therefore D = \ell_{max} \pm \ell_{max}\sqrt{\frac{K_s}{C_{s0}+K_s}}$$

D cannot exceed ℓ_{max}, why?

$$C_s = \frac{DK_s}{\ell_{max}-D} \quad \text{..... } C_s \to \overset{\text{-ve or}}{\infty} \text{ if } D \geq \ell_{max}$$

(-ve if $D > \ell_{max}$)

$$\therefore D = \ell_{max} - \ell_{max}\sqrt{\frac{K_s}{C_{s0}+K_s}}$$

$$\therefore D_{max\,prod} = \ell_{max}\left[1-\sqrt{\frac{K_s}{C_{s0}+K_s}}\right]$$

What about oxygen consumption?

OTR ≡ oxygen transfer rate

$$OTR = Q_{O_2} C_c$$

↑ rate of oxygen demand per mass of cells

$$\therefore \quad OTR = \underbrace{Q_{O_2} C_c}_{} = \underbrace{Y_{O_2/c} r_g} C_c$$

$$\therefore \quad OTR = Y_{O_2/c} r_g C_c$$

Also, $OTR = k_L a \left(C_{O_2}^* - C_{O_2}\right)$

- k_L: mass transfer velocity, units ≡ $\frac{m}{s}$ (or mass transfer coeff.)
- a: interfacial area (m^2)

$$\therefore \quad k_L a \left(C_{O_2}^* - C_{O_2}\right) = Y_{O_2/c} r_g C_c$$

X — X — X

$$\frac{dC_c}{dt} = r_G - r_D$$ ---- insert equations depending on type of regime

For substrate,

$$-r_S = Y'_{S/C} r_G + Y'_{S/P} r_P + m C_c$$

If $Y'_{S/C}$ & $Y'_{S/P}$ cannot be calculated, we lump them together as:

$$-r_S = Y_{S/C} r_G + m C_c$$

For product, one has to find out what type of production mode is involved
e.g. growth vs. non-growth

Consider brewing of ethanol:

we know 4 conditions, these are

→ no toxins involved

$$\therefore \quad r_D = k_D C_c$$

→ product becomes inhibitory

$$\therefore r_G = \left\{1 - \frac{C_p}{C_p^*}\right\}^n \left[\frac{\mu_{max} C_s C_c}{K_s + C_s}\right]$$

→ product is growth associated

$$\therefore r_p = Y_{P/C}\, r_G$$

→ $Y'_{S/C}$ & $Y'_{S/P}$ cannot be measured

$$\therefore \frac{dC_c}{dt} = \left\{1 - \frac{C_p}{C_p^*}\right\}^n \left[\frac{\mu_{max} C_s C_c}{K_s + C_s}\right] - k_D C_c \quad \hookrightarrow (1)$$

$$\frac{dC_s}{dt} = -Y_{S/C}\, r_G - m C_c$$

$$\therefore \frac{dC_s}{dt} = -Y_{S/C}\left\{1 - \frac{C_p}{C_p^*}\right\}^n \left[\frac{\mu_{max} C_s C_c}{K_s + C_s}\right] - m C_c \quad \hookrightarrow (2)$$

$$\& \quad \frac{dC_p}{dt} = Y_{P/C}\left\{1 - \frac{C_p}{C_p^*}\right\}^n \left[\frac{\mu_{max} C_s C_c}{K_s + C_s}\right] \rightarrow (3)$$

(1), (2) & (3) must be solved numerically.

Constants ≡ μ_{max}, K_s, m, C_p^*, n

* determined experimentally
* known for many strains