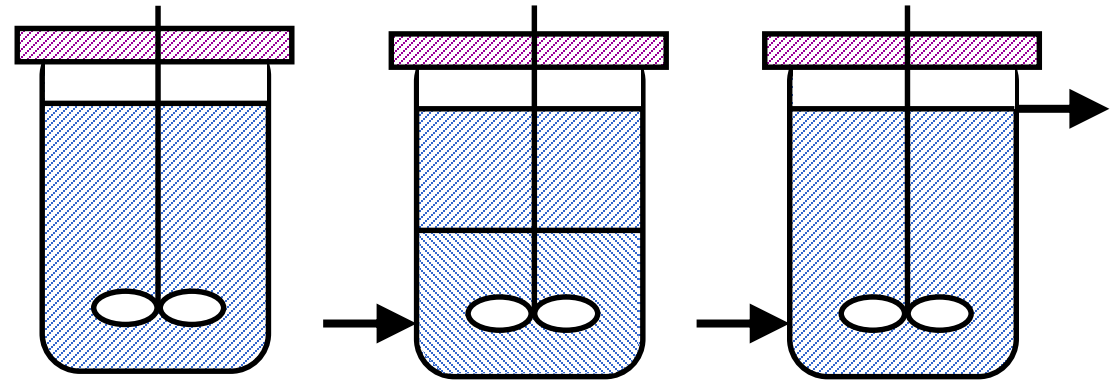
Bioprocesses → Bioreactors

From previous kinetic theory of specific rates $\mathbf{q_i}$: $_{\mu}$

- q_S (or) μ completely determines the **microbial behavior**
- q_S (or) μ must be controlled at an **optimal value μ_{opt}**

Generic type of process bioreactors:



Batch
2-12 days
Industry

Fed batch
1 – 3 days
Industry

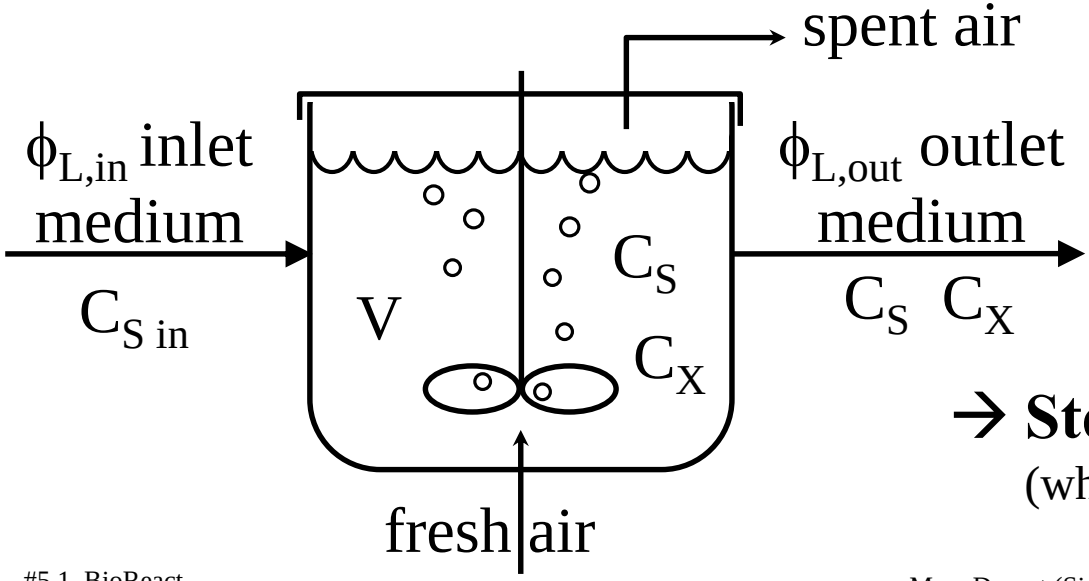
Continuous
(**Chemostat**)
10 - 100 days
Laboratory

1. Batch **$\mu = \mu^{max}$** and $q_S = q_S^{max}$ → μ is **not** controlled
2. **Chemostat** **μ can be controlled** as $\mu = D$ (Dilution rate) at μ^{opt}
3. Fed batch r_S is controlled by (C_S and inflow)

Chemostat process (1)

DEFINITION: Parameters - State variables of Chemostat bioprocess

Parameters			State variables
reactor	operator	micro-organism	
V $\phi_{L,out}$	$\phi_{L,in}$ $C_{S,in}$	q_S^{max} μ^{max} Y_{SX}^{max} m_S, K_S	C_S C_X



Main properties of chemostat:

- **$\mu = D$ Dilution rate** (?=1/HRT=1/SRT?)
- **C_S and C_X are independently manipulated** (by manipulating transport of substrate and biomass to and from the bioreactor)
- It's the (basic) **experimental tool** to study under controlled conditions :
  - **Microbial kinetics** $q_S(C_S)$, $q_P(\mu)$
  - **Stoichiometry of bioprocesses**

Complex transient dynamics!

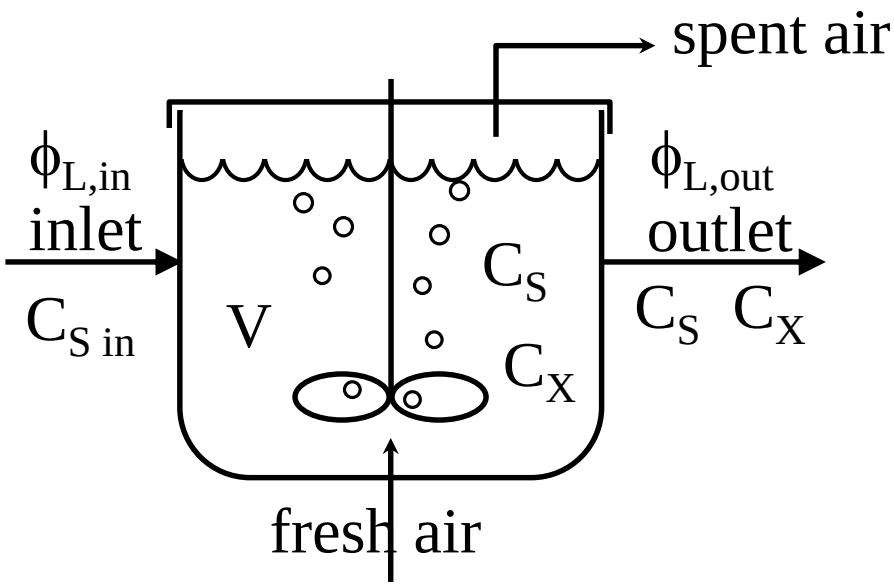
Constant $\phi_{L,in}$, $\phi_{L,out}$, C_S, C_X, Vol.

→ **Steady State ≡ Dynamic Equilibrium**
(where all state variables appear stable within time)

Chemostat process (2)

OPERATION of Chemostat bioprocess

1. A **bioreactor CSTR type** (Continuously Stirred Tank Reactor) (sterilized?) is filled with (sterile?) **growth medium** solution containing substrate $C_{S,\,in}$ N- and P-source, K^+, Mg^{2+}, salts, nutrients, vitamins, trace elements and chosen T, pH
2. Provide electron acceptor O_2, by **starting aeration and mixing**



3. **Inoculate** by adding a small amount of micro-organism (inoculum)
4. **Start feeding** the medium inflow (often $\phi_{L,\,in}$ and $\phi_{L,out}$ are nearly equal, not always)
5. **Wait until a Steady State** ≡ S.S. ≡ Dynamic equilibrium, is achieved

Chemostat process - Steady State (μ=D)

At S.S. mass balance for C_X → $\frac{d(VC_x)}{dt} = Q_{L,in}C_{x,in} - Q_{L,out}C_x + r_xV$

At S.S. mass balance for C_S → $\frac{d(VC_s)}{dt} = Q_{L,in}C_{s,in} - Q_{L,out}C_s + r_sV$

From mass balances: $r_x = \frac{Q_{L,out}C_x}{V}$ $\quad (-r_s) = \frac{Q_{L,in}C_{s,in} - Q_{L,out}C_s}{V}$

- $\mathbf{r_X}$ follows from experimentally measured data and the biomass mass balance
- **μ** comes from r_X and measured C_X, as $r_X = \mu \cdot C_X$

Thus : $\mu = \frac{r_x}{C_x} = \frac{Q_{L,out}}{V} \overset{def}{=} D : Dilution\ rate$

By choosing D, the operator fixes μ. Because μ (=D) is chosen, **all other q_i-values are chosen** from stoichiometric coupling (see chapter on kinetics).

Chemostat process - Steady State (C_S)

Using C_X mass balance → Calculate C_S residual substrate in bioreactor **(only limited by substrate)**

From kinetic expression for q_S [Herbert-Pirt Eq. and q_S hyperbolic kinetic] C_S and μ are linked,with Y_{SX}^{max}, m_S , q_S^{max} , K_S:

Thus μ can be expressed as $\mu = f(C_S)$ and $C_S = f(\mu)$…

$$q_S^{max} \cdot \frac{C_S}{k_S + C_S} = -q_S = \frac{1}{Y_{SX}^{max}} \mu + m_S$$

$$q_S^{max} Y_{SX}^{max} C_S = \left(\mu + m_S Y_{SX}^{max}\right).\left(k_S + C_S\right)$$

$$\mu = \frac{C_S\left(q_S^{max} Y_{SX}^{max} - m_S Y_{SX}^{max}\right) - m_S Y_{SX}^{max} k_S}{\left(k_S + C_S\right)} \quad with \quad q_S^{max} = \frac{1}{Y_{SX}^{max}} \mu^{max} + m_S$$

$$\mu = \frac{C_S \mu^{max} - m_S Y_{SX}^{max} k_S}{\left(k_S + C_S\right)} \quad with \quad C_S^{min} = \frac{m_S Y_{SX}^{max} k_S}{\mu^{max}}$$

$$q_S^{max} Y_{SX}^{max} C_S = \left(\mu + m_S Y_{SX}^{max}\right).\left(k_S + C_S\right)$$

$$C_S = \frac{\left(\mu + m_S Y_{SX}^{max}\right).k_S}{\left(q_S^{max} Y_{SX}^{max} - \left(\mu + m_S Y_{SX}^{max}\right)\right)} with \quad \mu^{max} = q_S^{max} Y_{SX}^{max} - m_S Y_{SX}^{max} \quad and \mu = D$$

$$C_S = \frac{m_S Y_{SX}^{max} k_S + D k_S}{\left(\mu^{max} - D\right)} \quad with \quad C_S^{min} = \frac{m_S Y_{SX}^{max} k_S}{\mu^{max}}$$

$$\mu = \mu^{max} \frac{C_S - C_S^{min}}{C_S + K_S} \quad ; C_S^{min} = m_s K_s Y_{sx}^{max} / \mu^{max}$$

$$C_S = \frac{C_S^{min} + K_S \frac{D}{\mu^{max}}}{1 - \frac{D}{\mu^{max}}}$$

Chemostat process - Steady State (C_S)

Thus, C_S residual substrate :

$$C_S = \frac{C_S^{min} + K_S \frac{D}{\mu^{max}}}{1 - \frac{D}{\mu^{max}}}$$

- **Is independent of $C_{S,in}$!!!**

- **Depends only on: - Microbial characteristics**
  - D dilution rate

??? How ???

$$\mu = \mu^{max} \frac{C_S - C_S^{min}}{C_S + K_S} \quad ; C_S^{min} = m_s K_s Y_{sx}^{max} / \mu^{max}$$

$$\mu = \frac{r_x}{C_x} = \frac{Q_{L,out}}{V} \overset{def}{=} D : Dilution\ rate$$

Specific growth rate depends only on C_S. During exponential batch growth, or very high C_S residual substrate, C_S^{min} becomes negligible, according "Monod" growth.

Chemostat process - Steady State (C_X)

Using C_S → Calculate C_X

Calculate r_S from measurements using C_S mass balance $(-r_s) = \frac{Q_{L,in}C_{s,in} - Q_{L,out}C_s}{V}$

Thus: $$(-r_s) = (\alpha.C_{s,in} - C_s).D \quad with \quad \alpha = \frac{Q_{L,in}}{Q_{L,out}}, D = \frac{Q_{L,out}}{V}$$

As $r_s = q_s.C_x \rightarrow C_x = \frac{(\alpha.C_{s,in} - C_s).D}{(-q_s)}$ as $Y_{sx} = \mu / q_s$

Thus C_X expression depends on $C_{S,in}$:
As C_S = f (D), Y_{SX} = f(μ) = f (D)

$$C_x = Y_{sx}\left(\alpha.C_{s,in} - C_s\right)$$

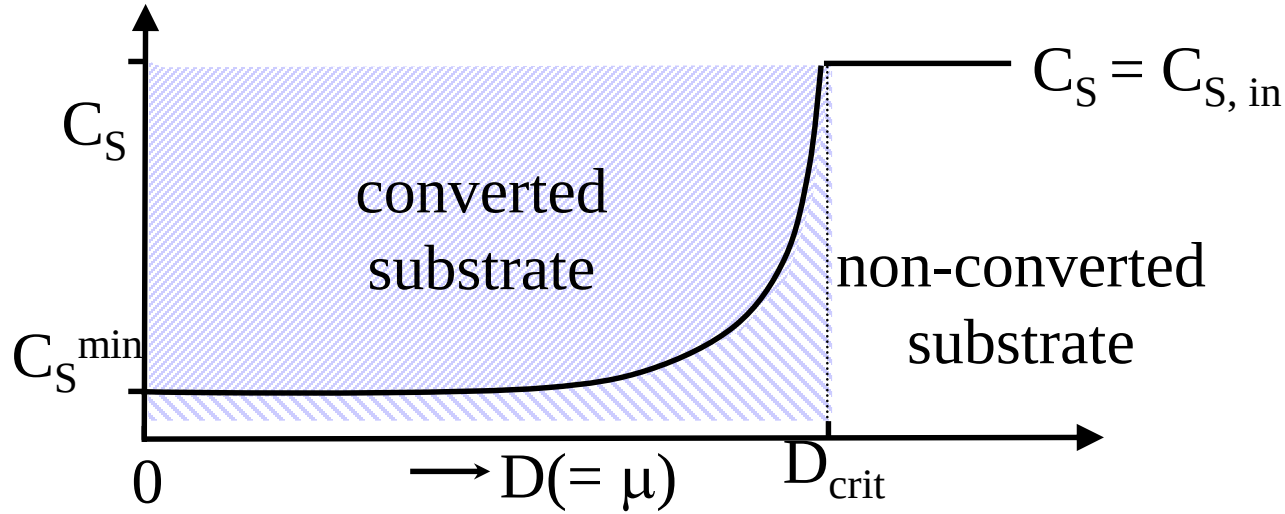
Mostly $C_S \approx 0$ for $D << \mu^{max}$

(Again, ONLY for exclusively substrate limited condition)

as $Y_{sx} = \mu / (-q_s)$ $C_s = \frac{C_S^{min} + K_S \frac{D}{\mu^{max}}}{1 - \frac{D}{\mu^{max}}}$

Chemostat process - Steady State (D_{crit})

$$C_S = \frac{C_S^{min} + K_S \frac{D}{\mu^{max}}}{1 - \frac{D}{\mu^{max}}}$$



With:

$C_{S,in}$ order of 10000; C_s^{min} order 1; K_S order of 10 [mgCOD.L^{-1}]

D_{crit} is the maximal dilution rate D (i.e. μ), achievable in the chemostat. It occurs when the maximal C_S is achieved: $C_S = C_{S,in}$.

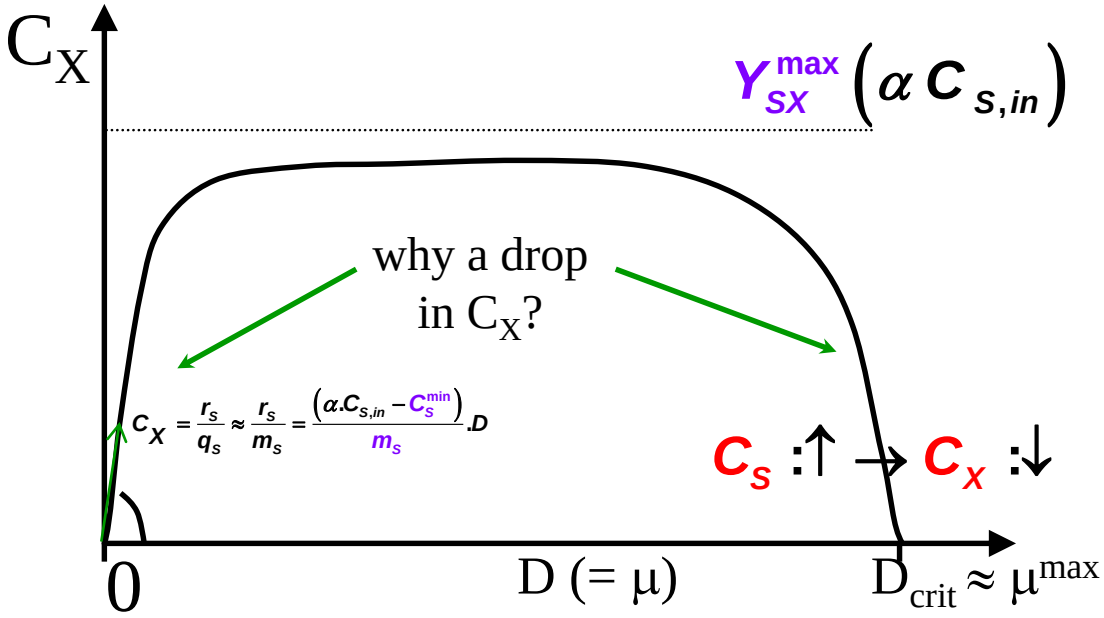
$$D_{crit} = \mu^{max} \cdot \frac{\left(C_{S,in} - C_S^{min}\right)}{K_S + C_{S,in}} \approx \mu^{max}$$

Washing out of the bioreactor occurs when the applied dilution rate exceeds the maximum specific growth for a given $C_{S,in}$. This happens when $D = D_{crit}$.

Chemostat process - Steady State (D)

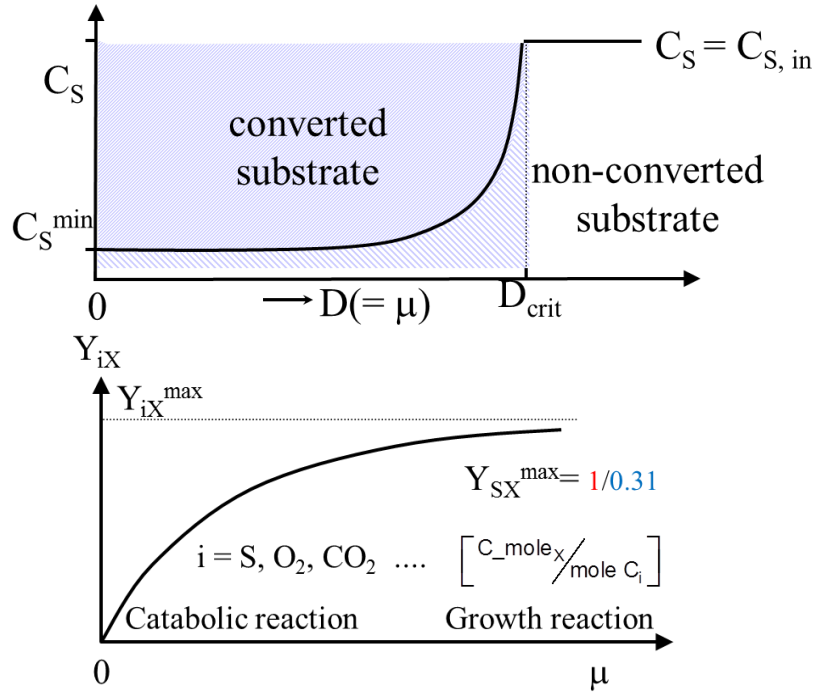
From: $C_X = Y_{SX}\left(\alpha.C_{S,in} - C_S\right)$

As Y_{SX} and C_S depends on D → C_X = f(D)



Maintenance!

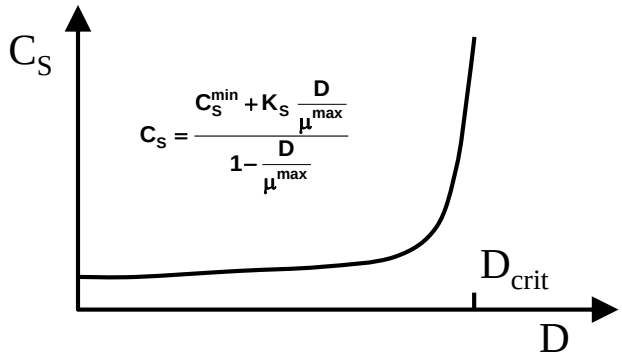
Substrate leaks! (by dilution)

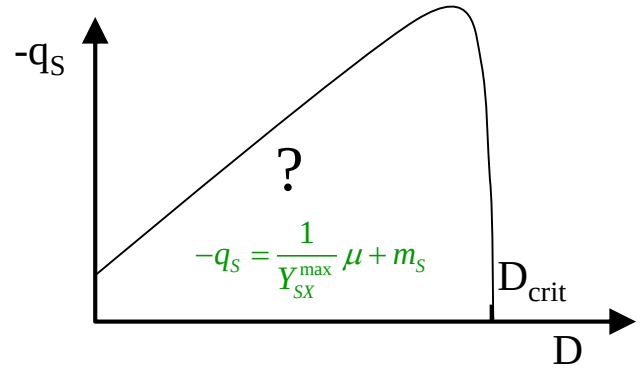


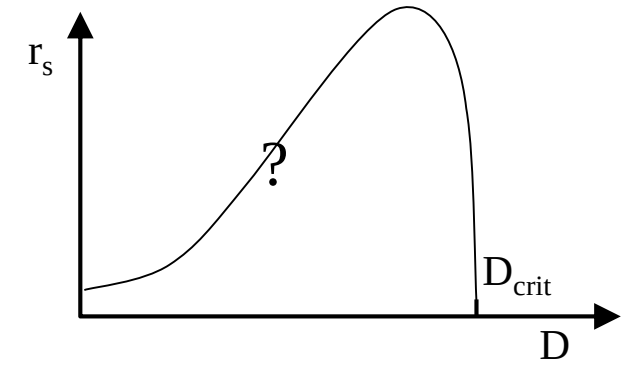
As biomass C_X depends on the overall C_S consumption :

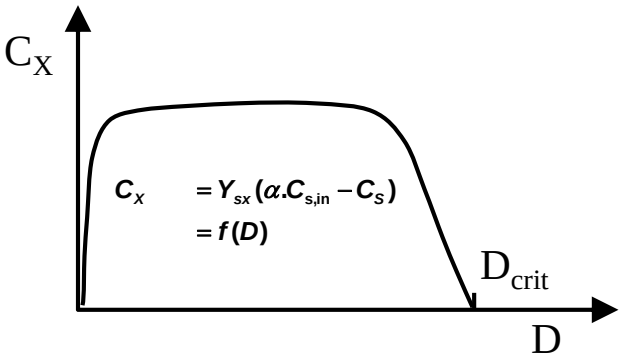
- At low D, low μ, low C_S, maintenance drives C_S consumption instead of growth…
- When D ≈ D_{crit}, the growth cannot consume all the available substrate, which leaks impair C_X growth…

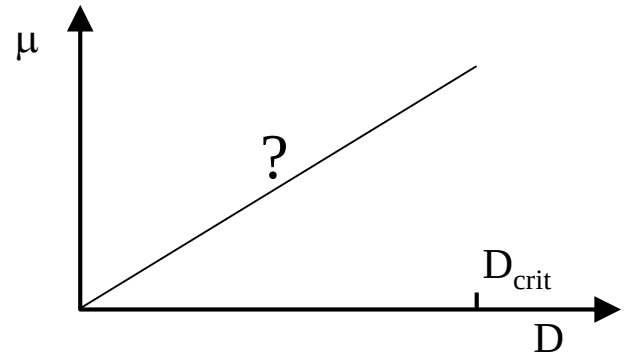
Chemostat - Behavior

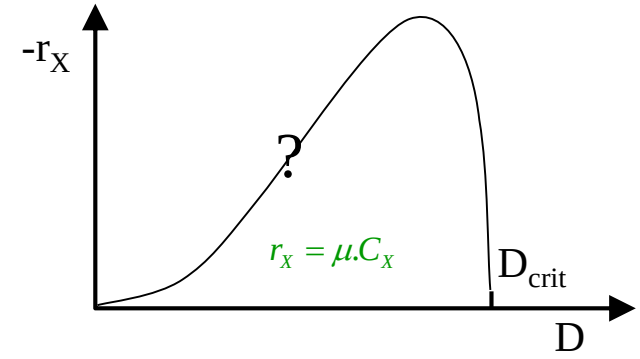


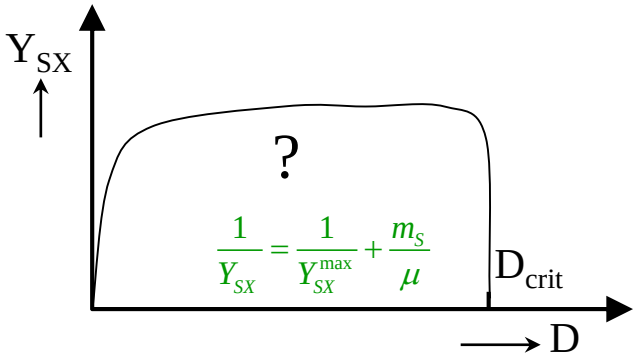


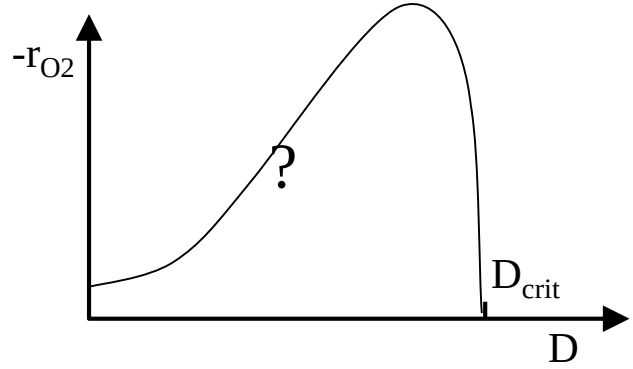


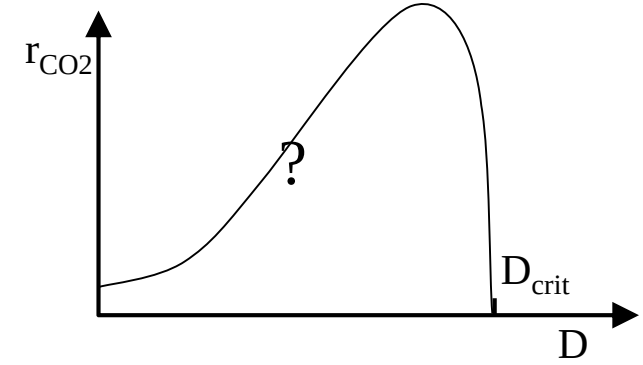






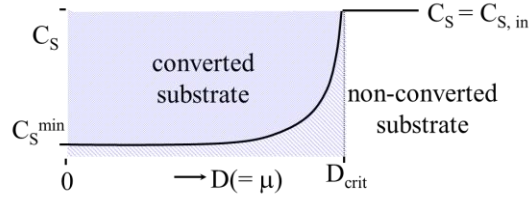






Chemostat - Applications

1. **Kinetic studies**



By choosing different D (= μ) values, and waiting for Steady State, as $C_S = f(D)$, one obtains Y_{sx}^{max} , m_S, q_S^{max} , K_S by linear or non linear fitting

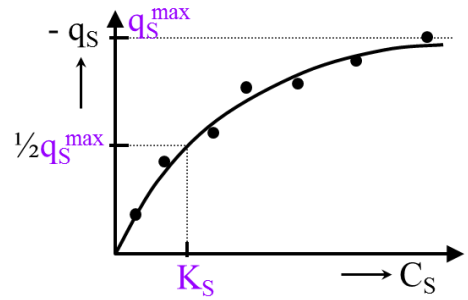
Lineweaver-Burk linearisation

$$\frac{1}{q_S} = \frac{k_S}{q_S^{max}} \cdot \frac{1}{C_S} + \frac{1}{q_S^{max}}$$

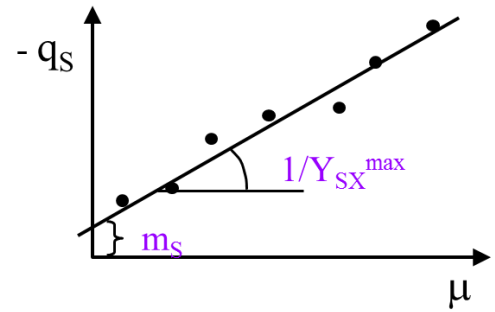
Hanes-Woolf linearisation

$$\frac{C_S}{q_S} = \frac{1}{q_S^{max}} . C_S + \frac{k_S}{q_S^{max}}$$

Hyperbolic equation $q_S = f(C_S)$



Herbert-Pirt equation $q_S = f(\mu)$



2. **Physiological studies** of microorganisms by change in substrate, electron acceptor, N-source or type of limitation …
3. **Waste water treatment purification** (+)/(-) ???
4. **Industrial fermentation** (not widely applied : low C_X, low C_P, biomass loss in outflow, microbial selection for non-producing mutants)

Chemostat – Optimisation (D_{opt})

What D-value = D_{opt} where r_S and r_X are maximal?

(which may be combined with economic parameters)

r_S = kg $S_{converted} \cdot m^{-3} \cdot h^{-1}$

From substrate mass balance and assuming $\alpha = 1$: $r_S = D \cdot (C_{S,in} - C_S)$

According, $C_S = f(D)$ graph, r_S has a maximum at higher D, therefore assuming m_S negligible → $C_S^{min} \approx 0$

Thus $C_S = \dfrac{K_S}{\frac{\mu^{max}}{D} - 1}$; $r_S = DC_{S\,in} - DK_S \times \dfrac{D}{\mu^{max} - D}$

D_{opt} follows from differentiation where $\dfrac{dr_S}{dD} = 0$

Using MathCAD

$$\text{Find}(D) \rightarrow \left[\frac{1}{2 \cdot (Csin + 1)} \cdot \left[2 \cdot Csin + 2 + 2 \cdot (Csin + 1)^{\frac{1}{2}} \right] \cdot mumax \quad \frac{1}{2 \cdot (Csin + 1)} \cdot \left[2 \cdot Csin + 2 - 2 \cdot (Csin + 1)^{\frac{1}{2}} \right] \cdot mumax \right]$$

Chemostat – Wash-out dynamics

Assume that D is close to $\mathbf{D_{crit}}$, and μ close to μ^{max} under Steady State conditions

→ What happens if D is increased to D' above μ^{max} ?
The biomass $\mathbf{C_X}$ **will be washed out** of the bioreactor.

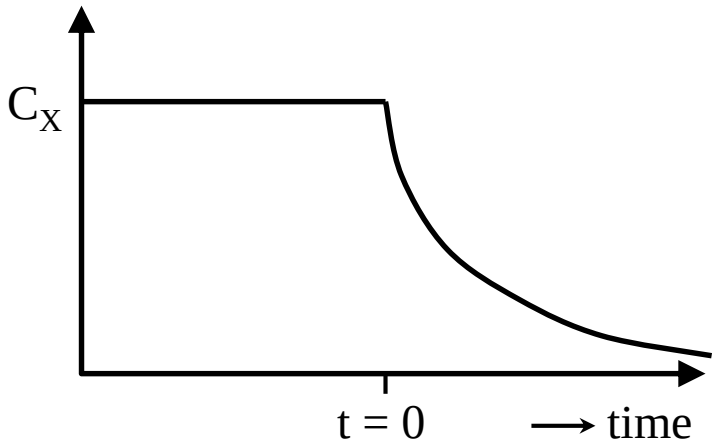
$$C_X(t) = C_{X(t=0)} * exp^{-(D' - \mu^{max})t}$$

$$or \quad ln \frac{C_X}{C_{X(t=0)}} = -(D' - \mu^{max})t$$

From biomass mass balance

$$\frac{dC_X}{dt} = \mu C_X - D'C_X$$

with $\mu \gg \mu^{max}$; $\mu^{max} - D' < 0$ *and cst*



→ **Washout curve can give μ^{max}!!!**
(Only under **non limited growth** C_S, C_{O2}, etc...)

Very useful for short μ^{max} = 1/HRT = D)

One Steady State is normally more than 3-5 HRT! If $1/\mu^{max}$ is about 3 weeks (methanogen), 3 months are required for 1 S.S.!