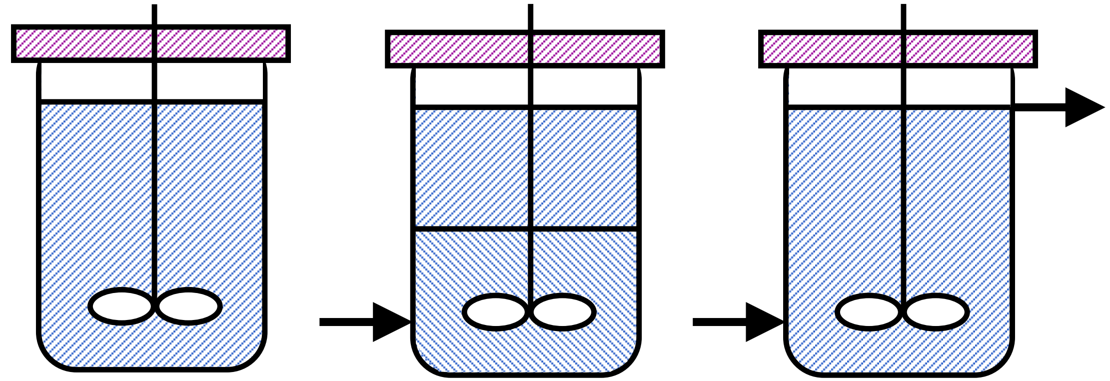
Bioprocesses → Bioreactors

Generic type of process bioreactors:

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

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



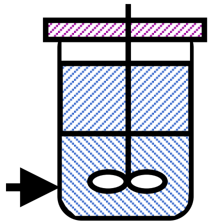
Batch
2-12 days
Industry

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 µ = D (Dilution rate) at μ^{opt}
3. **Fed batch** — $\mathbf{r_S}$ **is controlled** by (C_S and inflow rate)

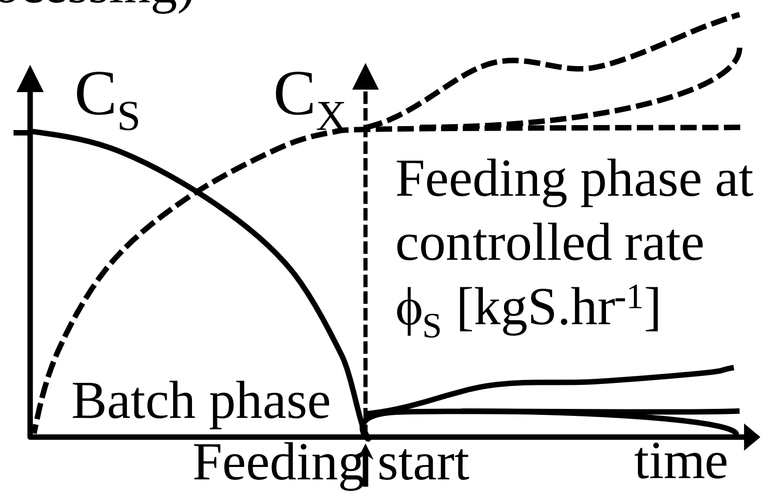
Fed batch Fermentation



Substrate transport IN
But not OUT
No biomass transport

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

- Low C_S → no toxicity / osmotic problems
- High C_X → high C_P → easier DSP (Down Stream Processing)
- Better biomass stability than chemostat



Key for fed batch calculations

- Herbert-Pirt Eq. → r_X
- Biomass balance → C_X

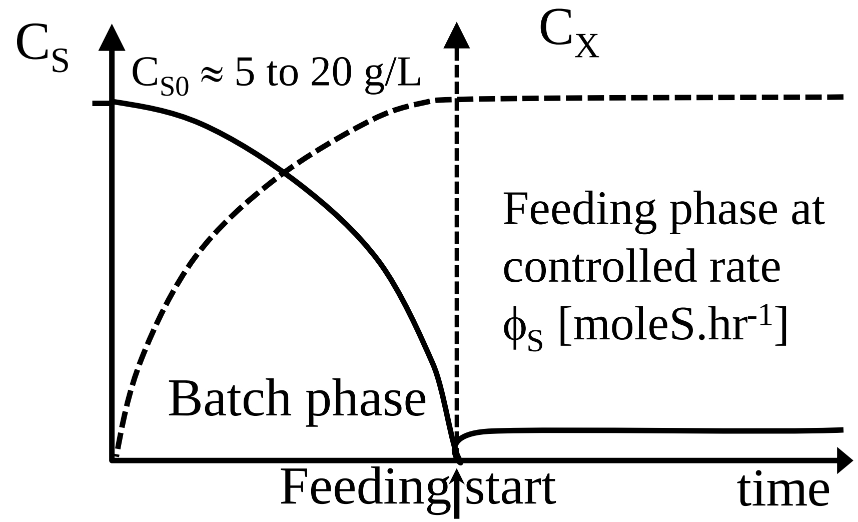
$$-r_S = \frac{\phi_S}{V}$$

→ Substrate feeding strategies

1. ϕ_S = constant
2. $\mu = \mu^{opt}$ (by control of C_S maximal q_P or Y_{SP})
3. Substrate feeding rate ϕ_S is determined by other known reactor limitations (O_2, heat, …)

Fed batch Fermentation: Assumptions…

After an initial batch, C_S is low, C_x is high.
Feeding phase starts with **high C_{Sin} load**,
by adjusting rate ϕ_S [moleS.hr^{-1}],
limited substrate conditions are maintained
with **low C_S** ≈ 1 to 50 mg/l
→ **Pseudo steady state for C_S (low)**



What about Volume changes?
Mass balance in reactor: dM/dt = Σ IN and OUT flow [kg.hr^{-1}]
= Feeding – water evaporation loss

As: - ρ_{water} = 1000 [kg.m^{-3}], most of reactor bulk mass is mass of water
- At high C_{Sin} load, evaporation loss almost compensates feeding

→ dM/dt ≈0 ; As most of the reactor bulk mass is water,
We can consider Mass pseudo steady state…
→ **Pseudo steady state of volume V (≈ Cst)**

$$\frac{dM}{dt} = 0 \rightarrow \frac{dV}{dt} = 0$$

Fed batch calculations

Calculation of C_X, C_S, C_P rates
assuming **pseudo-steady state for C_S (low and cst):**

$$-q_S = \frac{(-r_S)}{C_X} = \frac{\varphi_S}{VC_X}$$

$$\frac{d(VC_X)}{dt} = r_X V = \mu (VC_X); \quad \mu = \frac{d(VC_X)}{dt} * \frac{1}{VC_X}$$

$$\frac{d(VC_P)}{dt} = q_P (V.C_X); \; q_P = \frac{d(VC_P)}{dt} * \frac{1}{VC_X}$$

Hence from measured V, C_X, C_P, ϕ_S → one calculates μ, q_S, q_P

For simplicity assuming **V ≈ cst**

Example: In a fed batch fermentation process, a substrate solution (250 g.L^{-1}) is fed into the reactor with a constant rate of 1800 [L.h^{-1}].

Time hours	Volume m^3	C_X kg/m^3	C_P kg/m^3
90	100	60	20
92	105	61	21

Calculate for **average time** 91 h

ϕ_S, rates : μ, q_S, q_P

$$R_X = \Delta X/\Delta t \; ; \; r_X = R_X/V \; ; \; q_X = r_X/c_X$$

$$-r_S = \frac{\phi_S}{V} \; ; \; \phi_S = Q_{in} * C_{in}$$

$$\phi_S = 1800*0.250 = 450 \text{ [kgS.h}^{-1}\text{]}$$

$$\mu = \frac{(61*105)-(60*100)}{(92-90)*(102.5)*(60.5)} = 0.0327 \text{ [h}^{-1}\text{]}$$

$$q_S = \frac{450}{(102.5)*(60.5)} = 0.0726 \text{ [kgS.kgX}^{-1}\text{.h}^{-1}\text{]}$$

$$q_P = \frac{(21*105)-(20*100)}{(92-90)*(102.5)*(60.5)} = 0.0165 \text{ [kgP.kgX}^{-1}\text{.h}^{-1}\text{]}$$

Biomass mass balance in fed batch

Assuming volumic pseudo steady state:
$V \approx cst$

$$\frac{d(VC_X)}{dt} = r_X V; \quad V = Cst; \quad \frac{dC_X}{dt} = r_X$$

Calculate r_X using substrate Herbert-Pirt
By eliminating r_S using $-r_S = \phi_S/V$

$$q_S = \frac{1}{Y_{SX}^{max}}\mu + m_S; \ -r_S = \frac{\phi_S}{V} = q_S.C_X$$

$$rX = \mu.C_X = Y_{SX}^{max}.(q_S - m_S).C_X = Y_{SX}^{max}.\left(\frac{\phi_S}{V.C_X} - m_S\right).C_X$$

$$\frac{dC_X}{dt} = \underbrace{Y_{SX}^{max}.\frac{\phi_S}{V}}_{\text{Growth}} - \underbrace{Y_{SX}^{max}(m_S).C_X}_{\text{Maintenance}}$$

Assuming **$V \approx Cst$ AND $\phi_S = Cst$** : Analytical solution for $C_X(t)$ in feeding phase is:

$$C_X(t) = Y_{SX}^{max}\frac{\varphi_S}{V} \times \frac{1 - \exp\left(-Y_{SX}^{max}(m_S)t\right)}{Y_{SX}^{max}(m_S)} + C_{X_0 Feed}\exp\left(-Y_{SX}^{max}(m_S)t\right)$$

t= time after feeding start; C_{X0feed} = C_X at start feeding

Analytical solution for $C_X(t)$ in feeding phase

Assuming V ≈ Cst AND ϕ_S = Cst

$$\frac{dC_X}{dt} = Y_{SX}^{max} \cdot \frac{\phi_S}{V} - Y_{SX}^{max}(m_S).C_X$$

$$\rightarrow \quad C_X(t) = Y_{SX}^{max}\frac{\varphi_S}{V} \times \frac{1-\exp\left(-Y_{SX}^{max}(m_S)t\right)}{Y_{SX}^{max}(m_S)} + C_{X_0 Feed}\exp\left(-Y_{SX}^{max}(m_S)t\right)$$

Initially: C_X is quite low, maintenance can be neglected C_X increases linearly with time

For $t \ll 1/\left(-Y_{SX}^{max}(m_S)\right)$

$$\Rightarrow \frac{1-\exp\left(-Y_{SX}^{max}(m_S)t\right)}{Y_{SX}^{max}(m_S)} \approx t$$

$$\Rightarrow \exp\left(-Y_{SX}^{max}(m_S)t\right) \approx 1$$

$$\Rightarrow C_X(t) = C_{X_0 feed} + Y_{SX}^{max}\frac{\varphi_S}{V}t$$

Later: C_X increases, more and more, and substrate required for maintenance also.

End: almost all substrate is consumed for maintenance

For $t \gg 0$

$$\Rightarrow \exp(\ldots) \rightarrow 0$$

$$\Rightarrow C_X(t) \rightarrow \frac{\varphi_S / V}{(-m_S)}$$

Analytical solution for $C_X(t)$ in feeding phase

Assuming $V \approx$ Cst AND ϕ_S = Cst

Using $C_X(t)$ analytical solution $C_X(t) = Y_{SX}^{max} \frac{\varphi_S}{V} \times \frac{1 - \exp\left(-Y_{SX}^{max}(m_S)t\right)}{Y_{SX}^{max}(m_S)} + C_{X_0 Feed} \exp\left(-Y_{SX}^{max}(m_S)t\right)$

Thus in feeding phase $r_S(t)$, $r_X(t)$, $\mu(t)$ and $q_S(t)$ are:

$$-r_S = \frac{\phi_S}{V}$$

$$r_X(t) = \frac{dC_X}{dt} = Y_{SX}^{max} \underbrace{\frac{\varphi_S}{V}}_{= \text{cst}} - \underbrace{Y_{SX}^{max} m_S C_X(t)}_{\text{Analytical solution}}$$

$$\mu(t) = \frac{r_X(t)}{C_X(t)}$$

$r_X(t)$: decreases

$C_X(t)$: increases

→ μ strongly decreases

$$q_S(t) = \frac{r_S(t)}{C_X(t)}$$

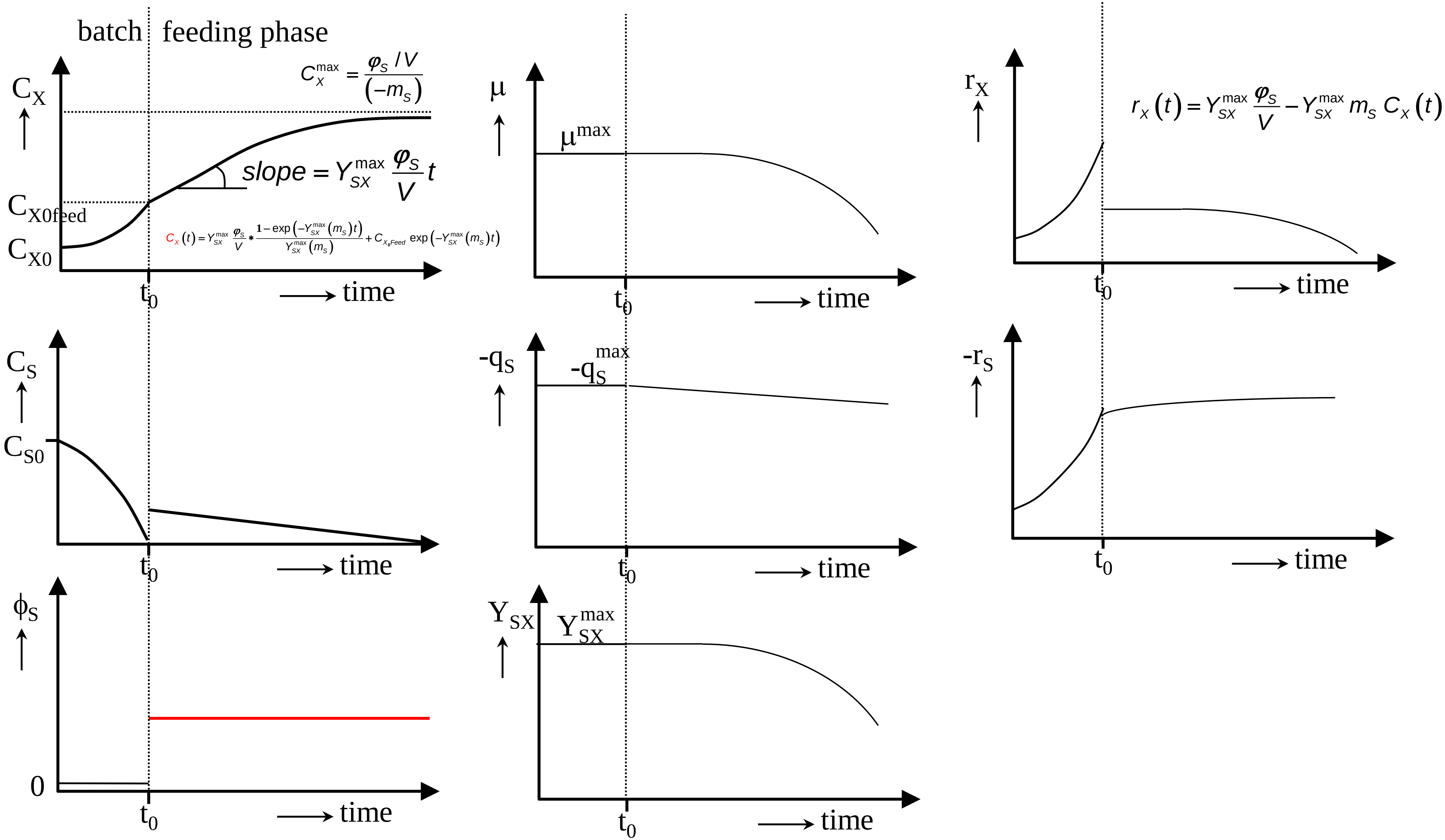
$r_S(t)$: cst

$C_X(t)$: increases

→ q_S decreases (but not to 0)

C_S slowly decreases

Fed batch behavior: ϕ_S = cst



Fed batch behavior: $\mu=\mu^{opt}=cst$

How do C_X, C_S, μ, q_S, Y_{SX}, r_X, r_S, ϕ_S change with time in feeding phase?

If $\mu = \mu^{opt} = cst \rightarrow q_i$, Y_{ij} are all constant (see kinetic chapter)

$\rightarrow q_S = cst = q_S^{opt}$ from Herbert Pirt Eq.

$\rightarrow C_S = cst$ from q_S hyperbolic Eq.

$$\frac{dC_X}{dt} = r_X(t) = \mu^{opt} C_X(t)$$

Assuming $V \approx Cst$…

$$r_i(t) = q_i^{opt} C_X(t)$$

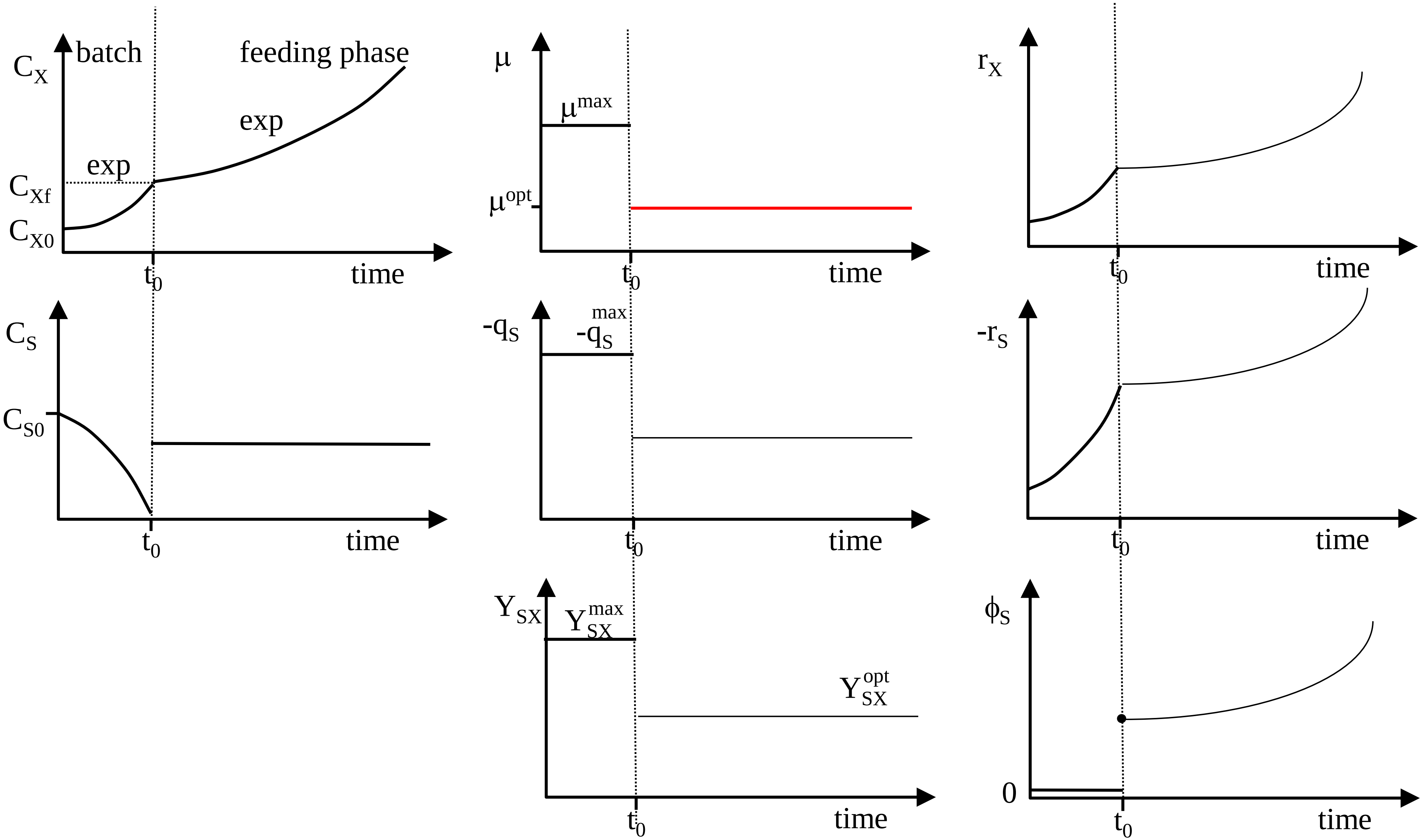
Thus, like in a batch: C_X biomass increases exponentially

All r_i rates increase exponentially

With an increasing feeding: $\phi_S(t) = (-r_S).V$

until $\phi_S(t) = \phi_S(t_0) * \exp(\mu^{opt}.t)$

Fed batch behavior: $\mu = \mu^{opt}$



Fed batch behavior: ϕ_S = cst

Including Product formation

Biomass concentration curve change as Herbert-Pirt equation changes:

$$-r_S = \left(\frac{1}{Y_{SX}^{\max}}.\mu + \frac{1}{Y_{SP}^{\max}}.q_P + m_S \right).C_X = \frac{1}{Y_{SX}^{\max}}.r_X + \frac{1}{Y_{SP}^{\max}}.r_P + m_S.C_X$$

Assuming $q_P = f(\mu)$, linear $q_P = a + b.\mu$

$r_P = a.C_X + b.r_X$.

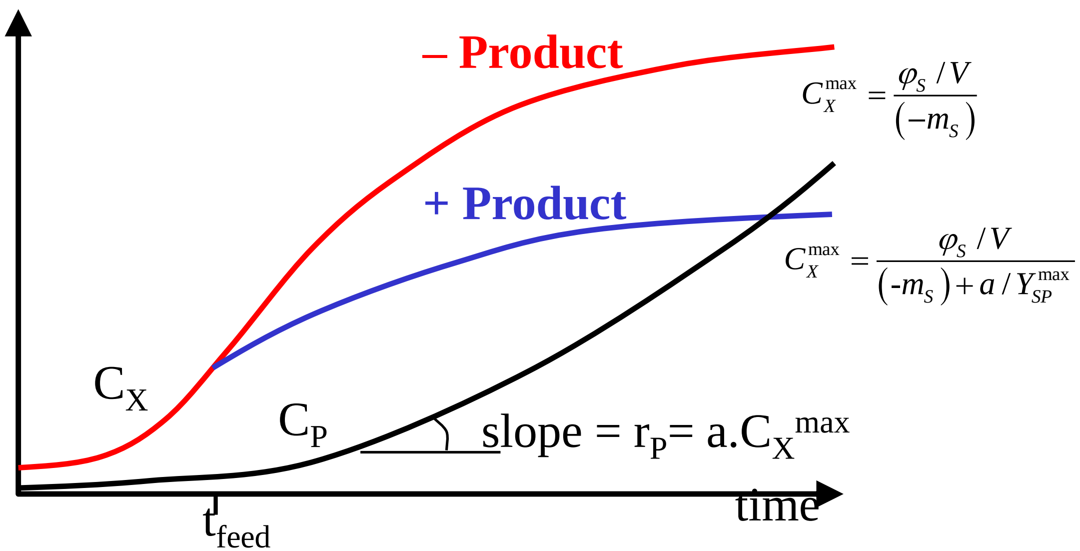
$$-r_S = \left(\frac{1}{Y_{SX}^{\max}} + \frac{b}{Y_{SP}^{\max}} \right) r_X + \left(\frac{a}{Y_{SP}^{\max}} + (-m_S) \right) C_X$$

Using $-r_S = \phi_S/V$ in biomass mass balance:

$$\frac{dC_X}{dt} = Y_{SX}^{\max}.\frac{\phi_S}{V} - Y_{SX}^{\max}(-m_S).C_X$$

(Without product)

$$\frac{dC_X}{dt} = \left(\frac{1}{\frac{1}{Y_{SX}^{\max}} + \frac{b}{Y_{SP}^{\max}}} \right) \frac{\varphi_S}{V} - \left(\frac{\frac{a}{Y_{SP}^{\max}} + (-m_S)}{\frac{1}{Y_{SX}^{\max}} + \frac{b}{Y_{SP}^{\max}}} \right) C_X$$



Same solution structure as before

For a on b quite small, q_P very small the same solution is obtained.