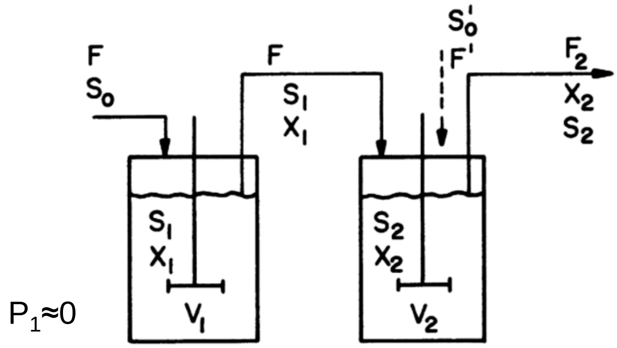
Multi-stage Chemostat System

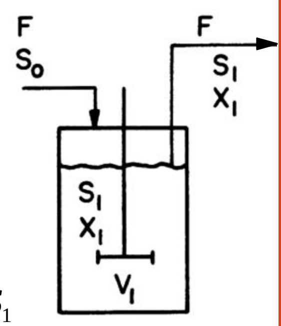
- In some fermentations, the growth and product-formation steps need to be separated, since optimal conditions for each step are different. e.g. secondary metabolite, culture of genetically engineered cells.



Growth stage Product formation stage

At steady state, V_n, X_n, S_n, P_n in the reactor of each stage don't change with time.

Multi-stage Chemostat System



X_0=0, V_i, = constant.

Stage 1: cell growth condition, $k_d = 0$, $q_p = 0$

Cell mass: $FX_0 - FX_1 + \mu_1 X_1 V_1 = V_1 \dfrac{dX_1}{dt}$

At steady state $\boxed{\mu_1 = D_1 (1 - \dfrac{X_0}{X_1})}$

Limiting substrate: $FS_0 - FS_1 - V_1 \dfrac{\mu_1 X_1}{Y^M_{X/S}} = V_1 \dfrac{dS_1}{dt}$

At steady state $\boxed{S_1 = S_0 - \dfrac{\mu_1 X_1}{D_1 Y^M_{X/S}}}$

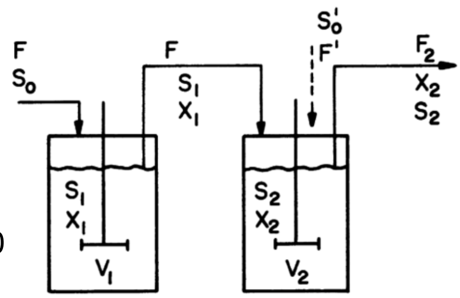
> μ_1 , μ_2 ... and μ_n are net specific growth rates in Stage 1, 2,..., and n, recspectively.
> When $k_d = 0$, they are equal to the respective specific gross growth rates in each stage.

Multi-stage Chemostat System

Stage 2 – product formation conditions, $k_d = 0$, F' = 0 ➔ $F_2 = F$

Cell mass: $$FX_1 - FX_2 + \mu_2 X_2 V_2 = V_2 \frac{dX_2}{dt}$$

At steady state $$\boxed{\mu_2 = D_2 (1 - \frac{X_1}{X_2})}$$ where $D_2 = \frac{F}{V_2}$



$P_1 \approx 0$

P_2

V_2 is constant.

Multi-stage Chemostat System

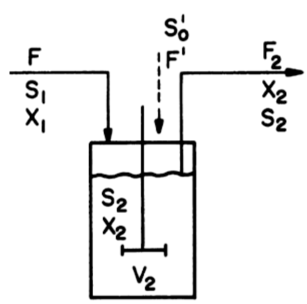
- **Stage 2 – product formation conditions, $k_d = 0$, F' = 0 → $F_2 = F$**

Limiting substrate: $$FS_1 - FS_2 - V_2 \frac{\mu_2 X_2}{Y^M_{X/S}} - V \frac{q_p X_2}{Y_{P/S}} = V_2 \frac{dS_2}{dt}$$

At steady state $$\boxed{S_2 = S_1 - \frac{\mu_2 X_2}{D_2 Y^M_{X/S}} - \frac{q_p X_2}{D_2 Y_{P/S}}}$$

Product: $$FP_1 - FP_2 + V_2 q_p X_2 = V_2 \frac{dP_2}{dt}$$

At steady state $$\boxed{P_2 = P_1 + \frac{q_p X_2}{D_2}}$$



Multi-stage Chemostat System

Stage n – product formation conditions, Kd=0, F'=0

Similarly, equations could be obtained for nth stage.

$$\mu_n = D_n(1 - \frac{X_{n-1}}{X_n})$$

$$S_n = S_{n-1} - \frac{\mu_n X_n}{D_n Y^M_{X/S}} - \frac{q_p X_n}{D_n Y_{P/S}}$$

$$P_n = P_{n-1} + \frac{q_p X_n}{D_n}$$

If μ_n (e.g. Monod model) and q_p are known functions, X_n , P_n, and S_n at nth stage could be determined by the above equations.

Multi-stage Chemostat System

- To determine the parameters (X, P) graphically when growth kinetics cannot be expressed analytically.
- With no additional streams added to the second or subsequent units, mass balance around the nth stage on cell, substrate and product yields

$$FX_{n-1} - FX_n + \mu_n X_n V_n = V_n \frac{dX_n}{dt}$$

$$\text{at steady state } \frac{dX_n}{dt} = 0, \qquad \mu_n X_n = D_n(X_{n-1} - X_n)$$

$$\text{Since } r_{x,n}(X_x, S_n) = \mu_n X_n, \qquad r_{x,n}(X_x, S_n) = D_n(X_n - X_{n-1})$$

$r_{x,n}$: cell growth rate at nth stage (g/l-h);

F: the volumetric flowrate (l/h);
D_n =F/V_n: the dilution rate at the nth stage (1/h);
V_n: the liquid volume of the nth chemostat (l)

Multi-stage Chemostat System

$$FP_{n-1} - FP_n + r_{p,n}V_n = V_n \frac{dP_n}{dt}$$

$$FS_{n-1} - FS_n + r_{s,n}V_n = V_n \frac{dS_n}{dt}$$

Similarly at steady state

$$r_{p,n}(X_x, S_n) = D_n(P_n - P_{n-1})$$

$$r_{s,n}(X_x, S_n) = \frac{1}{Y_{X/S}} r_{x,n}(X_x, S_n) = D_n(S_n - S_{n-1})$$

$r_{p,n}$: product formation at nth stage (g/l-h)

$r_{s,n}$: substrate consumption at nth stage (g/l-h)

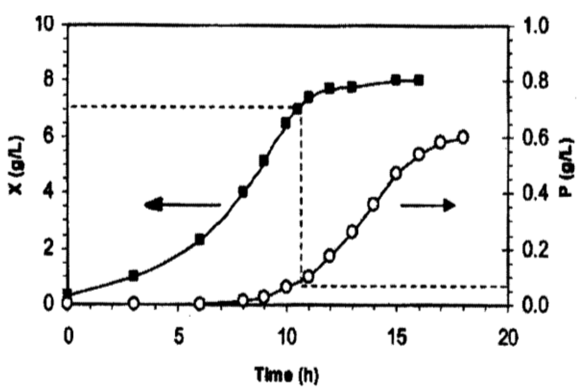
Multi-stage Chemostat System

To determine X and P graphically:

For example, in a two-stage chemostat system, the first tank is 600 l and the second one is 900 l. The flowrate is 100l/h, determine the concentrations of cells and product in each of the tank.

Solution: - Get kinetics data (X~t, P~t) from the batch culture.

originating from (P_{n-1}, 0).



Get the kinetics data of X~t and P~t from batch culture and plot

Get dX/dt~X from the kinetics data and plot

Determine X_1 and P_1 in the first stage:

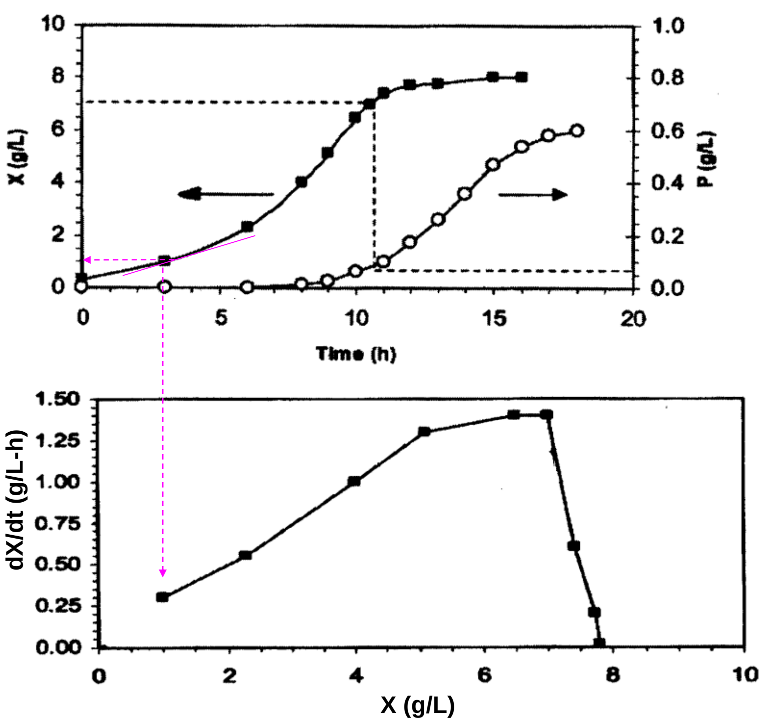
On the graph of dX/dt~X, draw a line of $y = D_1 (X_1 - X_0)$	$D_1 = F/V_1$, $X_0 = 0$ in the feed	X_1 is determined from the intersection.

On the graph of X,P~t, P_1 is determined by the X_1 at the respect time

Determine X_2 and P_2 in the second stage:

Plot the graph of dP/dt~P from the kinetics data of P,	draw a line of $y = D_2(P_2 - P_1)$, $D_2 = F/V_2$	P_2 is determined from the intersection.

On the graph of X,P~t, X_2 is determined by the P_2 at the respect time.



Get the kinetics data of X~t and P~t from batch culture and plot

Get dX/dt~X from the kinetics data and plot

Determine X_1 and P_1 in the first stage:

On the graph of dX/dt~X, draw a line of $y = D_1 (X_1 - X_0)$	$D_1 = F/V_1$, $X_0 = 0$ in the feed	X_1 is determined from the intersection.

On the graph of X,P~t, P_1 is determined by the X_1 at the respect time

Determine X_2 and P_2 in the second stage:

Plot the graph of dP/dt~P from the kinetics data of P,	draw a line of $y = D_2(P_2-P_1)$, $D_2 = F/V_2$	P_2 is determined from the intersection.

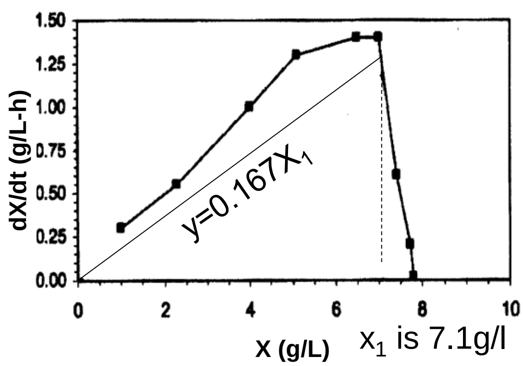
On the graph of X,P~t, X_2 is determined by the P_2 at the respect time.

Multi-stage Chemostat-Graphical Solution

- Get data of dX/dt~X from the kinetics data in the batch culture and plot.

$$r_{x,n}(X_{x,}S_n)$$
$$= D_n(X_n - X_{n-1})$$

$$y = D_1(X_1 - X_0)$$
$$= \frac{\text{F}}{\text{V}_1}(X_1 - X_0)$$
$$= \frac{100\text{l/h}}{600\text{l}}(X_1 - 0)$$
$$= 0.167 X_1$$



x_1 is 7.1g/l

-Determine the x_1 in the first stage of chemostat.

On the graph of dX/dt~X, draw a line of $y = D_1(X_1 - X_0)$ originating from $(X_{0\,,}\ 0)$. x_1 can be found from the intersection.

Get the kinetics data of X~t and P~t from batch culture and plot

Get dX/dt~X from the kinetics data and plot

Determine X_1 and P_1 in the first stage:

On the graph of dX/dt~X, draw a line of $y = D_1 (X_1 - X_0)$

$D_1 = F/V_1$, $X_0 = 0$ in the feed

X_1 is determined from the intersection.

On the graph of X,P~t, P_1 is determined by the X_1 at the respect time

Determine X_2 and P_2 in the second stage:

Plot the graph of dP/dt~P from the kinetics data of P,

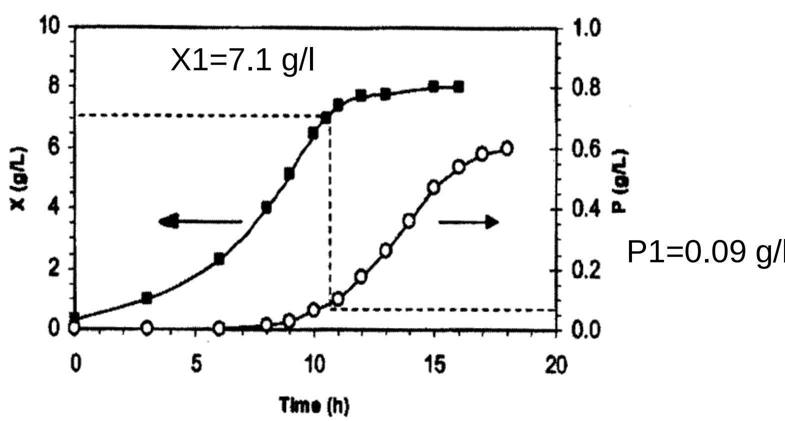
draw a line of $y = D_2(P_2-P_1)$, $D_2 = F/V_2$

P_2 is determined from the intersection.

On the graph of X,P~t, X_2 is determined by the P_2 at the respect time.

Multi-stage Chemostat-Graphical Solution

- From the graph of kinetics data get the P_1 in respect with the X_1.



Get the kinetics data of X~t and P~t from batch culture and plot

Get dX/dt~X from the kinetics data and plot

Determine X_1 and P_1 in the first stage:

On the graph of dX/dt~X, draw a line of $y = D_1 (X_1 - X_0)$	$D_1 = F/V_1$, $X_0 = 0$ in the feed	X_1 is determined from the intersection.

On the graph of X,P~t, P_1 is determined by the X_1 at the respect time

Determine X_2 and P_2 in the second stage:

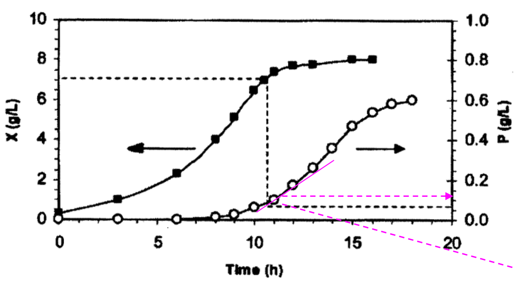
Plot the graph of dP/dt~P from the kinetics data of P,	draw a line of $y = D_2(P_2 - P_1)$, $D_2 = F/V_2$	P_2 is determined from the intersection.

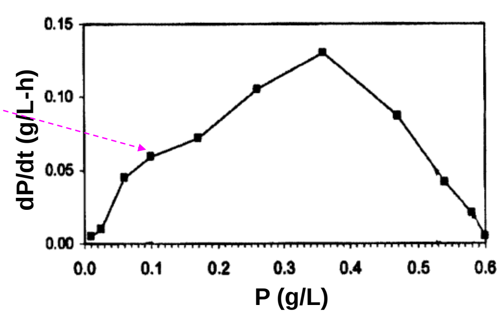
On the graph of X,P~t, X_2 is determined by the P_2 at the respect time.

Multi-stage Chemostat-Graphical Solution

Determine the X_2 and P_2 For the second stage.

- get dP/dt ~ P from the kinetics data in the batch culture and plot.





Get the kinetics data of X~t and P~t from batch culture and plot

Get dX/dt~X from the kinetics data and plot

Determine X_1 and P_1 in the first stage:

On the graph of dX/dt~X, draw a line of $y = D_1 (X_1 - X_0)$	$D_1 = F/V_1$, $X_0 = 0$ in the feed	X_1 is determined from the intersection.

On the graph of X,P~t, P_1 is determined by the X_1 at the respect time

Determine X_2 and P_2 in the second stage:

Plot the graph of dP/dt~P from the kinetics data of P,	draw a line of $y = D_2(P_2-P_1)$, $D_2 = F/V_2$	P_2 is determined from the intersection.

On the graph of X,P~t, X_2 is determined by the P_2 at the respect time.

Multi-stage Chemostat-Graphical Solution

$$r_{p,n}(X_x, S_n \ldots) = D_n(P_n - P_{n-1})$$

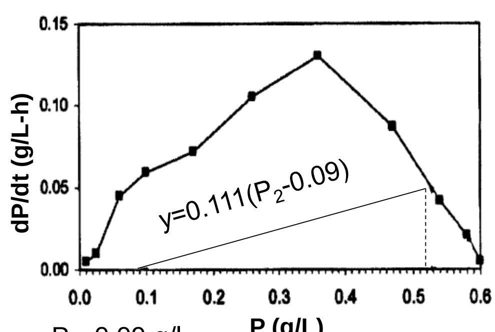
on the graph of dP/dt ~ P, draw a line of $y = D_2(P_2 - P_1)$

$$y = D_2(P_2 - P_1)$$

$$= \frac{F}{V_2}(P_2 - P_1)$$

$$= \frac{100 l/h}{900 l}(P_2 - 0.09 g/l)$$

$$= 0.111(P_2 - 0.09 g/l)$$



P_1=0.09 g/l

P_2=0.53 g/l

P_2 can be determined from the intersection.

Get the kinetics data of X~t and P~t from batch culture and plot

Get dX/dt~X from the kinetics data and plot

Determine X_1 and P_1 in the first stage:

On the graph of dX/dt~X, draw a line of $y = D_1 (X_1 - X_0)$	$D_1 = F/V_1$, $X_0 = 0$ in the feed	X_1 is determined from the intersection.

On the graph of X,P~t, P_1 is determined by the X_1 at the respect time

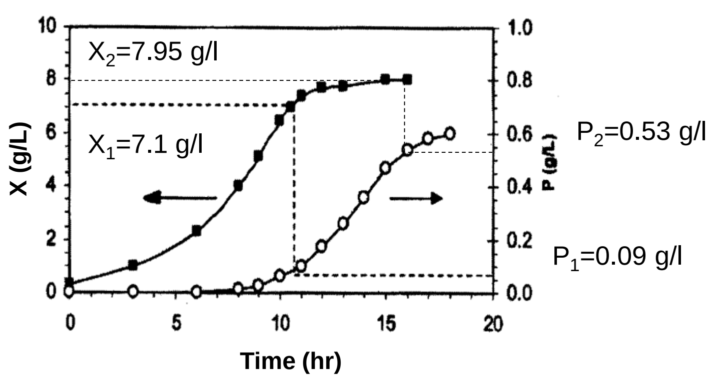
Determine X_2 and P_2 in the second stage:

Plot the graph of dP/dt~P from the kinetics data of P,	draw a line of $y = D_2(P_2-P_1)$, $D_2 = F/V_2$	P_2 is determined from the intersection.

On the graph of X,P~t, X_2 is determined by the P_2 at the respect time.

Multi-stage Chemostat-Graphical Solution

- From the graph of kinetics data get the X_2 in respect with the P_2



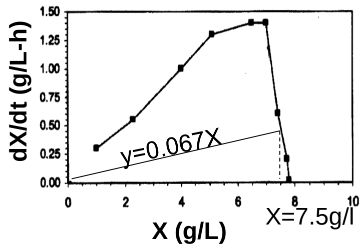
- Similarly, Xn and Pn can be determined for the nth stage of reactor.

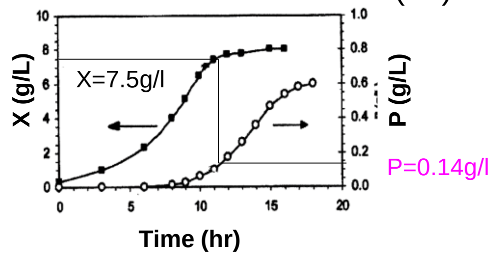
Multi-stage Chemostat-Graphical Solution

In summary, the first tank is 600 l and the second one is 900 l.
The flowrate is 100l/h, X_1=7.1 g/l, P_1=0.09 g/l; X_2=7.95 g/l, P_2=0.53 g/l

If one stage chemostat is used with the same total working volume i.e. 1500 l as that in the above example.
The flowrate is also 100l/h. What is the achieved concentrations of the cells and the product?

$D=F/V=100/1500=0.067\ (h^{-1})$





Product concentration can be increased by using multistage chemostats instead of single stage with the same total reactor working volume.