BIOREACTOR ENGINEERING
Chapter 6
Culture Kinetic Study of Modifying Continuous Fermentation

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Chapter Description

- Topic Outcome
  - Perform culture kinetic calculation on modifying continuous bioreactor operation

- References

  - Doran, P.M. (2013) Bioprocess Engineering Principles. Elsevier.
  - Liu, S. (2013) Bioprocess Engineering: Kinetics, Biosystem, Sustainability and Reactor Design. Elsevier.
  - Rao, D.G. (2010) Introduction to Biochemical Engineering. McGraw Hill.

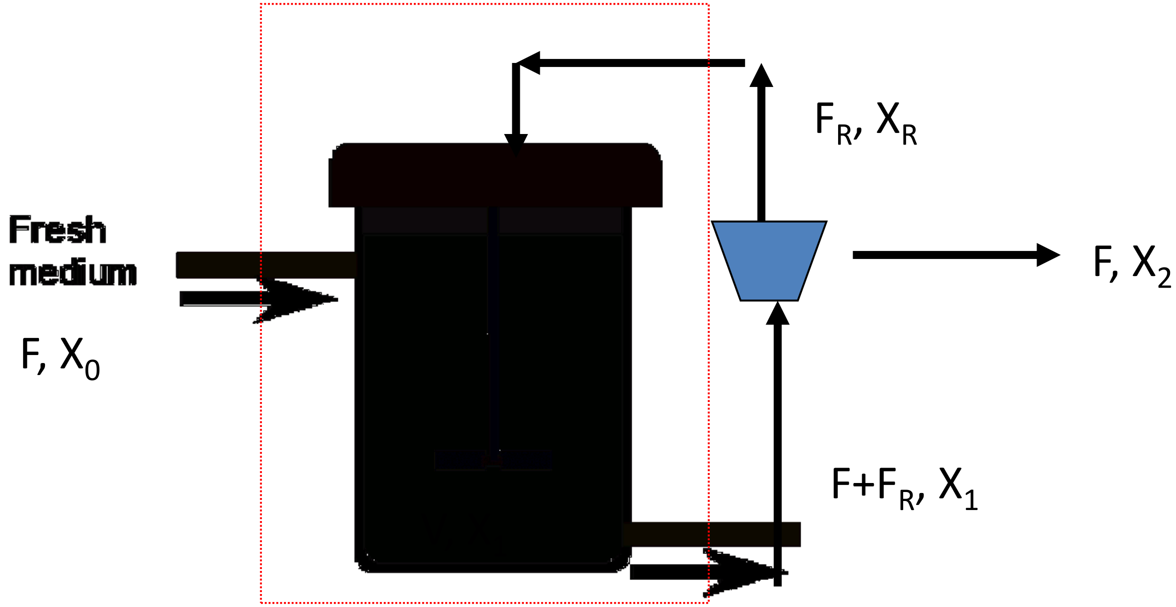
Topic Outline

- Chemostat with Cell Recycle
- Multistage Chemostat System

Chemostat with Cell Recycle

- Chemostats cannot be operated if $\mu_{max} < D$. Higher dilution rates can be achieved with recycle.
- Cells in the effluent is recycled back to the reactor in order to keep the cell concentration higher than the normal steady state level.

F - nutrient flow rate
V - culture volume
X_1 - X concentration in reactor
X_2 - X concentration in effluent
X_R - X concentration in recycle
F_R - recycle flow rate



Chemostat with Cell Recycle

- Material balance on the cell mass around the fermenter (cell maintenance and death are assumed to be small)

Cell in medium feed + Cell in recycle stream – Cell out + Cell growth = Cell accumulation

Please write the formula = ?

Define:

recycle ratio, a = F_R/F

concentration factor, C = X_R / X_1

- So, $$\frac{F}{V}X_0 + \frac{\alpha F C}{V}X_1 - (1+\alpha)\frac{F}{V}X_1 + \mu X_1 = \frac{dX_1}{dt}$$

Chemostat with Cell Recycle

- During steady state, $\frac{dX_1}{dt} = 0$ and $D = \frac{F}{V}$, assuming medium feed is sterile ($X_o = 0$)

$$\mu = (1 + \alpha - \alpha C)D = [1 + \alpha(1 - C)]D$$

- if C > 1 (concentration of cells) then $\alpha(1 - C) < 0$ then $\mu < D$
- A chemostat can be operated at dilution rates higher than the specific growth rate when cell recycle is used

Chemostat with Cell Recycle

- From Monod equation: $\mu = \mu_{max} \frac{S}{K_s + S}$

- Substitute Monod equation, solve:

$$S = \frac{K_S D[1 + \alpha(1 - C)]}{\mu_{\max} - D[1 + \alpha(1 - C)]}$$

Chemostat with Cell Recycle

- Material balance on the growth limiting substrate S around the fermenter

Substrate in feed stream + Substrate in recycle stream – Substrate out – Substrate consumed = Substrate accumulation

Please write the formula = ?

Define: recycle ratio, a = F_R/F

$$\frac{F}{V}S_0 + \frac{\alpha F}{V}S - (1+\alpha)\frac{F}{V}S - \frac{\mu X_1}{Y_{X/S}} = \frac{dS}{dt}$$

Chemostat with Cell Recycle

- During steady state, $\frac{dS}{dt} = 0$ and $D = \frac{F}{V}$

  $X_1 = \frac{D}{\mu} Y_{X/S} (S_0 - S)$ → (1)

- From previous, we have: $\mu = (1 + \alpha - \alpha C) D$ → (2)
- (2) in (1): $X_1 = \frac{Y_{X/S}(S_0 - S)}{(1 + \alpha - \alpha C)}$

- When cell recycle is used, cell concentration in a chemostat is increased by a factor of $\frac{1}{(1 + \alpha - \alpha C)}$

Chemostat with Cell Recycle

- Exercise 1

Multistage Chemostat System

- Applicable to fermentation in which growth and product formation need to be separated into stages (with different optimal condition; different T, pH, and limiting nutrient)
- Particular for secondary metabolite production (culture of genetically engineered cells), uses an inducible promoter to control production of the target protein.

Multistage Chemostat System

1st stage:

- From single stage chemostat, during steady state, medium feed is sterile, cell maintenance and death are assumed to be small
- From cell balance: $\mu_1 = D_1$ $\mu_1 = \frac{\mu_{max} S_1}{K_s + S_1}$
- Rearrange: $S_1 = \frac{K_S D_1}{\mu_{max} - D_1}$ where $D_1 = \frac{F}{V_1}$
- From substrate balance: $X_1 = Y_{X/S}(S_0 - S_1)$

Multistage Chemostat System

2nd stage: (without additional feed)

- Material balance on the cell mass, X

Cell in – Cell out + Cell growth = Cell accumulation

Please write the formula = ?

- During steady state, $\frac{dX_2}{dt} = 0$ and $D_2 = \frac{F}{V_2}$

$$\mu_2 = D_2\left(1 - \frac{X_1}{X_2}\right)$$

$$\mu_2 = \frac{\mu_{max} S_2}{K_s + S_2}$$

- where $\frac{X_1}{X_2} < 1, \mu_2 < D_2$

Multistage Chemostat System

2nd stage: (without additional feed)

- Material balance on the growth limiting substrate, S

Substrate in – Substrate out – Substrate consumed = Substrate accumulation

Please write the formula = ?

- During steady state, $\frac{dS_2}{dt} = 0$ and $D_2 = \frac{F}{V_2}$

- So, $S_2 = S_1 - \frac{\mu_2}{D_2} \frac{X_2}{Y_{X/S}}$ rearrange: $X_2 = \frac{D_2 Y_{X/S}}{\mu_2}(S_1 - S_2)$

Multistage Chemostat System

2nd stage: (without additional feed)

- Material balance on the product, P

Product in – Product out + Product synthesis = Product accumulation

Please write the formula = ?

- During steady state, $\frac{dP_2}{dt} = 0$ and $D_2 = \frac{F}{V_2}$

- No product from 1[st] stage, so: $P_2 = \frac{q_p X_2}{D_2}$

Multistage Chemostat System

- Exercise 2

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