BIOREACTOR ENGINEERING
Chapter 2
Culture Kinetic Study of Batch Fermentation

by
Chew Few Ne
Faculty of Chemical & Natural Resources Engineering
cfne@ump.edu.my

Chapter Description

- Topic Outcome
  - Perform calculation regarding culture kinetics of batch fermentation

- 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

- Kinetics of Cell Growth and Death
- Kinetics of Substrate Consumption
- Kinetics of Product Formation

Kinetics of Cell Growth and Death

- The volumetric rate of cell growth is described by:

$$r_X = \mu X$$

r_X = volumetric rate of cell growth (g /L .h)

X = cell concentration (g/L)

μ = specific growth rate (h^{-1})

- The volumetric rate of cell death is described by:

$$r_d = k_d X$$

r_d = volumetric rate of cell death (g /L .h)

k_d = specific death rate (h^{-1})

Kinetics of Cell Growth and Death

- At any time during fermentation,

cell accumulation = cell in – cell out + cell growth – cell death

$$\frac{dX}{dt} = \frac{F}{V}X_0 - \frac{F}{V}X + r_X - r_d$$

$\frac{dX}{dt}$ = rate of change of cell concentration (g /L .h)

X = cell concentration (g/L)

X_0 = cell centration in the feed (g/L)

t = time (h)

F = volumetric flow rate from the culture (L /h)

V = culture volume (L)

Culture Kinetic Study of Batch Fermentation by Chew Few Ne

Kinetics of Cell Growth and Death

- Assume no cells are fed in and removed from the culture
- During exponential phase: $k_d << \mu$
- Thus, $$\frac{dX}{dt} = \mu X$$
- The equation indicates that the rate of change of cell concentration is directly proportional to the cell concentration

Kinetics of Cell Growth and Death

- If μ is constant, integration yields: $lnX = lnX_i + \mu t$
- Thus, μ can be determined from the slope of the plot of ln X versus t.
- Time required to achieve certain cell density can be calculated by: $t = \frac{1}{\mu_{max}} ln \frac{X}{X_i}$
- The doubling time t_d is the time required for the cell concentration to double: $t_d = \frac{ln2}{\mu}$

Kinetics of Cell Growth and Death

- Exercise 1

Kinetics of Cell Growth and Death

- Assume no cells are fed in and removed from the culture
- During death phase: $k_d >> \mu$
- Thus, $\frac{dX}{dt} = -k_d X$

- If k_d is constant, integration yields: $\ln X = \ln X_i - k_d\, t$

Kinetics of Cell Growth and Death

- Exercise 2

Kinetics of Cell Growth and Death

- Substrate concentration will affect the growth rate in batch culture
- The μ depends on the substrate concentration
- The effect of S on μ can be described by Monod equation:

$$\mu = \mu_{max} \frac{S}{K_s + S}$$

μ = specific growth rate (h^{-1})
μ_{max} = maximum specific growth rate (h^{-1})
K_S = saturation/Monod constant (g/L)
S = substrate concentration (g/L)

- K_S expresses the affinity of the organism for the limiting substrate
  - High K_S= low affinity for the substrate

Culture Kinetic Study of Batch Fermentation by Chew Few Ne

Kinetics of Cell Growth and Death

- μ_{max} is achieved in the presence of non-limiting substrate concentration ($S >> K_S$): $\mu = \mu_{max}$
- If $K_S = S$: $\mu = \frac{\mu_{max}}{2}$
- Lineweaver-Burk plot can be used to determined μ_{max} and K_S , by plotting $\frac{1}{\mu}$ versus $\frac{1}{S}$

$$\frac{1}{\mu} = \frac{1}{S} . \frac{K_S}{\mu_{max}} + \frac{1}{\mu_{max}}$$

Kinetics of Cell Growth and Death

- Certain substrate maybe toxic at higher concentration, resulting in growth inhibition, μ
  - Very high concentration of nutrient →high osmotic stress → dehydration
  - Certain ions are toxic →inhibitory effect on certain key enzymes or structural components of the cell
  - Certain substrates (e.g., methanol, formaldehyde, and toluene) are extremely toxic (> 1 g/L) →damage cell membranes and react with the amino acid components of proteins.

Kinetics of Substrate Consumption

- Rate of substrate uptake during cell culture: $r_s = q_s X$

  r_s = volumetric rate of substrate consumption (g /L .h)

  X = cell concentration (g/L)

  q_s = specific rate of substrate uptake (h^{-1})

- Substrate are consumed to provide:
  - The necessary carbon, energy and structural components for cell growth
  - The product formation
  - The maintenance of cell viability (e.g., cell repair mechanisms, and substrate transport process)

Kinetics of Substrate Consumption

- Thus, the rate of substrate consumption:

$$r_s = \frac{r_X}{Y_{X/S}} + \frac{r_p}{Y_{P/S}} + mX$$

$$r_s = \left(\frac{\mu}{Y_{X/S}} + \frac{q_p}{Y_{P/S}} + m\right)X$$

$$\therefore q_s = \frac{\mu}{Y_{X/S}} + \frac{q_p}{Y_{P/S}} + m$$

where

$Y_{X/S}$ = the theoretical yield of cell from substrate

$Y_{P/S}$ = the theoretical yield of product from substrate

μ = specific growth rate (h^{-1}).

q_p = specific rate of product formation (h^{-1}).

m = maintenance coefficient (g substrate consumed per g cell dry weight per hour)

X = cell concentration (g/L)

Kinetics of Substrate Consumption

- At any time during fermentation,

Substrate accumulation = substrate in – substrate out – substrate for growth – substrate for product formation – substrate for maintenance

$$\frac{dS}{dt} = \frac{F}{V}S_0 - \frac{F}{V}S - r_s$$

where

$\frac{dS}{dt}$ = rate of change of substrate concentration (g /L .h)

F = volumetric flowrate of medium in and out of the bioreactor (L/h)

V = volume of the culture (L)

S_0, S = subtrate concentration going in and out of the culture (g/L)

Kinetics of Substrate Consumption

- Assume no substrate feed and substrate removal, the maintenance coefficient is very small and no products are formed, thus: $\frac{dS}{dt} = -\frac{\mu X}{Y_{X/S}}$
- The equation indicates that the rate of change of substrate concentration is directly proportional to the cell concentration
- Time required for a particular level of substrate conversion:

$$t = \frac{1}{\mu_{max}} \ln\left[1 + \frac{Y_{X/S}}{X_i}(S_i - S)\right]$$

- The specific rate of substrate consumption: $q_S = -\frac{1}{X}\frac{dS}{dt}$

Kinetics of Substrate Consumption

- q_s is usually not constant throughout batch culture.
- q_s can be obtained at any time from experimental data of substrate concentration versus time.
- To obtain q_s, divide the value for the slope ds/dt by the value of x at the time of measurement.

Kinetics of Product Formation

- Rate of product formation in cell culture: $r_p = q_p X$

  r_p = volumetric rate of product formation (g /L .h)
  X = cell concentration (g/L)
  q_p = specific rate of product formation (h^{-1})

- At any time during fermentation,

Product accumulation = product synthesis – product denaturation – product removal

$$\frac{dP}{dt} = q_P X - \beta \mathrm{P} - \frac{F}{V} P$$

$\frac{dP}{dt}$ = rate of change of product concentration (g /L .h)
q_p = specific rate of product formation (h^{-1})
X = cell concentration (g/h)
β = specific product denaturation rate (h^{-1})
P = Product concentration (g /L)
F = volumetric flow rate from the culture (L /h)
V = culture volume (L)

Culture Kinetic Study of Batch Fermentation by Chew Few Ne

Kinetics of Product Formation

- Assume the product is stable and not removed, thus: $\frac{dP}{dt} = q_P X$
- The equation indicates the rate of change of product concentration is directly proportional to the cell concentration
- Time required to achieve a particular product concentration:

$$t = \frac{1}{\mu_{max}} \ln \left[1 + \frac{\mu_{max}}{X_i q_p} (P - P_i) \right]$$

- The specific rate of product formation: $q_P = \frac{1}{X} \frac{dP}{dt}$

Kinetics of Product Formation

- q_p is usually not constant throughout batch culture.
- q_p can be obtained at any time from experimental data of product concentration versus time.
- To obtain q_p, divide the value for the slope dp/dt by the value of x at the time of measurement.

CREDITS

Special thanks to
- Prof. Dr. Tey Beng Ti
- Prof. Madya Dr. Rosfarizan binti Mohamad
- Dr. Farhan binti Mohd Said

Thanks to Dr. Rozaimi bin Abu Samah for proofreading the learning contents.