Biomass Production in Cell Cultures

Not everything that counts can be counted, and not everything that can be counted counts. *Albert Einstein*

Introduction

- When a small amount of livings cells is added to a liquid solution having essentials nutrients at suitable temperature and pH, the cells will grow.
- The growth processes have two different manifestations according to the morphology of the cells involved.
- For unicellular organisms which divides as they grow, increase in biomass (mass of living matter)is accompanied by increase in the number of cells present.
- Associated with cell growth are two other processes:
  - uptake of some material from the cell's environment and
  - release of metabolic end products into the surroundings.

Biomass and Cell cultures

- Biomass is the mass of living matter in a population of particular organisms in a particular area.

$$\text{Substrates} + \text{Cells} \longrightarrow \text{Extracellular products} + \text{More cells}$$

$$\sum S + X \longrightarrow \sum P + nX$$

Cell Growth and Kinetic Patterns

$$\text{Substrates} + \text{Cells} \longrightarrow \text{Extracellular products} + \text{More cells}$$

$$\sum S + X \longrightarrow \sum P + nX$$

- Cell materials serve as a catalyst.
- Substrates react to produce more cells and metabolic products.

Kinetic Patterns of Growth and Product Formation in Batch Fermentation

- Microbial products can be classified into:
  - Growth associated products
  - Non-Growth associated products
  - Mixed Growth associated products

Growth associated products

- produced simultaneously with microbial growth.
- The rate of product formation (q_p) is:

$$q_P = \frac{1}{X}\frac{dP}{dt} \tag{1}$$

$$q_P = Y_{P/X} \times \mu_g \tag{2}$$

X = cell mass concentration
μ_g = gross specific growth rate
$Y_{P/X} = \frac{\Delta P}{\Delta X}$ yield coefficient of product on mass cell

Non-growth associated products

- occurs during the stationary phase when growth rate is zero.

$$q_P = \beta = \text{constant} \tag{3}$$

Mixed growth associated products

- occur during the slow growth and stationary phases.

$$q_P = \alpha\mu_g + \beta \qquad \text{(Leudeking-Piret model)} \tag{4}$$

  α and β are constants.

- If $\alpha = 0$, the product is non-growth associated

$$q_P = \beta$$

- If $\beta = 0$, the product is growth associated.

$$q_P = \alpha\mu_g$$

$$\alpha = Y_{P/X}$$

Yield Coefficient and Maintenance Coefficient

Yield Coefficient

- Based on the amount of consumption of another material.
  For example, in a fermentation process, growth yield is;

$$Y_{X/S} = \frac{\Delta X}{\Delta S}$$

- Also based on other substrates or product formation.

$$Y_{X/O_2} = \frac{\Delta X}{\Delta O_2}$$

$$Y_{P/S} = \frac{\Delta P}{\Delta S}$$

X = cellular material formed
S = amount of substrate consumed
P = amount of product formed

- The yield coefficients, for example, for organisms growth aerobically on glucose are: $Y_{X/S} : 0.4 - 0.6\text{g/g}$ $Y_{X/O_2} : 0.9 - 1.4\text{g/g}$

Maintenance Coefficient

- Used to describe the specific rate of substrate uptake for cellular maintenance.

$$M = -\frac{[dS/dt]_m}{X}$$

- During the stationary phase in which a little external substrate is available, endogenous metabolism of biomass components is used for maintenance energy.
- Basically, cellular maintenance represents the energy expended to repair the damaged materials and components to derive the normal functioning cells.

Mass Balance on Substrate

- Consider a continuous stirred tank reactor with volume V to required to keep the contents inside the reactor well mixed.

  At the feed side:
  Let F be the flow rate of the feed, C_{if} be the concentration in the feed, S_f be the substrate concentration in the feed.
  In the reactor, the contents are well mixed and change to X_i and C_i with S as the substrate concentration.

  At the effluent side:

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

$$Y_{X/S} = \frac{\Delta X}{\Delta S}$$

Mass Balance on Substrate (2)

Substrate balance: Taking the mass balance at steady state (feed side = effluent side)

$$\frac{F}{V_R}(S_f - S) = Y_{S/X}\mu X \tag{5}$$

$$\frac{F}{V_R}(S_f - S) - Y_{S/X}\mu X = 0 \tag{6}$$

$$D(S_f - S) - \frac{\mu X}{Y_{X/S}} = 0 \tag{7}$$

$D =$ Dilution rate $= \frac{F}{V_R}$

Mass Balance on Substrate (3)

But

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

Therefore,

$$D(S_f - S) - \frac{\mu_{max} S X}{Y_{X/S}(K_S + S)} = 0 \tag{8}$$

Substrate balance:

$$D X_f = (D - \mu) X \tag{9}$$

$$D X_f + (\mu - D) X = 0 \tag{10}$$

$$D X_f + \left[\frac{\mu_{max} S}{K_S + S} - D\right] X = 0 \tag{11}$$

Mass Balance on Substrate (4)

Product balance:

$$D(P_f - P) + Y_{P/X}\mu X = 0 \tag{12}$$

$$P = P_f + \frac{Y_{P/X}\mu X}{D} \tag{13}$$

Growth of Microorganism in a Batch Reactor

- Involves addition of small amount of MOs or their spores (seed culture or inoculum) to a quantity of nutrient material in a suitable vessel.
- process is either aerobic or anaerobic.
- In aerobic fermentation, growth process requires the presence of molecular oxygen, the contents of the vessel are well stirred and aerated and later growth is allowed to proceed.

The general balance equation for fermenter is:
$\{\text{Rate of flow of material in}\} + \{\text{Rate of formation}\} - \{\text{Rate of flow of material out}\} = \text{Accumulation}$

Growth of Microorganism in a Batch Reactor (2)

- In a batch reactor, the flow in and out is both zero.
  {Rate of formation} = Accumulation

$$r_f \cdot V = \frac{dX}{dt} \cdot V \tag{14}$$

$$r_f = \frac{dX}{dt} \tag{15}$$

For Biomass:

$$r_f \cdot V = \mu V X = \frac{dX}{dt} \cdot V \tag{16}$$

- If S is considered to be a concentration of limiting substrate, then

$$r_S \cdot V = \frac{dS}{dt} \cdot V \tag{17}$$

Growth of Microorganism in a Batch Reactor (3)

Using $Y_{X/S} = \frac{\Delta X}{\Delta S}$

$$Y\frac{dS}{dt} = -\frac{dX}{dt} \tag{18}$$

$$Y\frac{dS}{dt} = -\mu X \tag{19}$$

$$Y \cdot \frac{dS}{dt} = -\mu X \tag{20}$$

$$-\frac{\mu X}{Y} = \frac{dS}{dt} \tag{21}$$

But

$$Y = Y_{X/S} = \frac{\Delta X}{\Delta S} = \frac{X - X_0}{S_0 - S} \tag{22}$$

$$S = S_0 - \frac{X - X_0}{Y_{X/S}} \tag{23}$$

Growth of Microorganism in a Batch Reactor (4)

Recall that $\mu = \frac{\mu_{max}[S]}{K_S+[S]}$

$$\frac{\mu_{max}[S]}{K_S + [S]} X = \frac{dX}{dt} \tag{24}$$

The condition of fermentation of culture after any time t is obtained by integrating using boundary conditions: $t = 0, X = X_0$ and $t = t, X = X$

$$\int_{X_0}^{X} \frac{K_S + [S]}{\mu_{max}[S]} \frac{dX}{X} = \int_0^t dt \tag{25}$$

Substituting Eq. (23) and integrating:

$$\frac{K_S Y + S_0 Y + X_0}{\mu_m ax(YS_0 + X_0)} ln\Big(\frac{X}{X_0}\Big) + \frac{K_S Y}{\mu_m ax(YS_0 + X_0)} \cdot ln\Big(\frac{YS_0}{YS_0 + X_0 - X}\Big) = t \tag{26}$$

Growth of Microorganism in a Batch Reactor (5)

For substrate concentration:

$$\frac{K_S Y + S_0 Y + X_0}{\mu_m ax(YS_0 + X_0)} ln\Big(1 + \frac{Y(S_0 - S)}{X_0}\Big) - \frac{K_S Y}{\mu_m ax(YS_0 + X_0)} \cdot ln\Big(\frac{S}{S_0}\Big) = t \qquad (27)$$

Continuous Culture of Microorganisms

- Growth of MOs is continuous and achieved as continuous chemical reactions that are carried out either in tubular fermenter or in a well-mixed vessel (Back mix fermenter).
- consider continuous stirred tank fermenter (CSTF) to be analogous to a continuous stirred tank reactor (CSTR).
- The CSTF can be operated as turbidostat, i.e. feed is metered in such a way that a constant biomass concentration is maintained.

The overall balance is:

$\{\text{Material flow in}\} + \{\text{Rate of formation by biochemical reaction}\} - \{\text{Material flow out}\} = \text{Accumulation}$

Continuous Culture of Microorganism

For biomass:

$$FX_f + r_{fx}V - FX = \frac{dX}{dt}V \tag{28}$$

For substrate:

$$FS_0 + r_S V - FS = \frac{dS}{dt}V \tag{29}$$

r_S is negative as substrate is consumed.
Recall that $D = \frac{F}{V_R}$

$$\frac{F}{V_R}X_f + r_{fx} - \frac{F}{V_R}X = \frac{dX}{dt} \tag{30}$$

$$DX_f + r_{fx} - DX = \frac{dX}{dt} \tag{31}$$

Continuous Culture of Microorganism (2)

At steady state, $\frac{dX}{dt} = 0$

$$DX_f + r_{fx} - DX = \frac{dX}{dt} \tag{32}$$

But $r_{fx} = \mu X$ (Malthus law)

$$D(X_f - X) = -\mu X \tag{33}$$

$$D = \frac{-\mu X}{X_f - X} \tag{34}$$

If the feed is sterile $X_f = 0$

$$D = \mu \tag{35}$$

$$D = \frac{\mu_{max} S}{K_S + S} \tag{36}$$

Continuous Culture of Microorganism (3)

$$S = \frac{DK_S}{\mu_{max} - D} \tag{37}$$

But

$$Y_{X/S} = \frac{X_f - X}{S_f - S} \tag{38}$$

$$X = X_f + Y_{X/S}(S_f - S) \tag{39}$$

Substituting Eq. (37) into Eq. (39), we have:

$$X = X_f + Y_{X/S}\left[S_f - \left(\frac{DK_S}{\mu_{max} - D}\right)\right] \tag{40}$$

For a sterile feed, $X_f = 0$

$$X = Y_{X/S}\left[S_f - \left(\frac{DK_S}{\mu_{max} - D}\right)\right] \tag{41}$$