PHEN 612

SPRING 2008

WEEK 4

LAURENT SIMON

Bioreactors

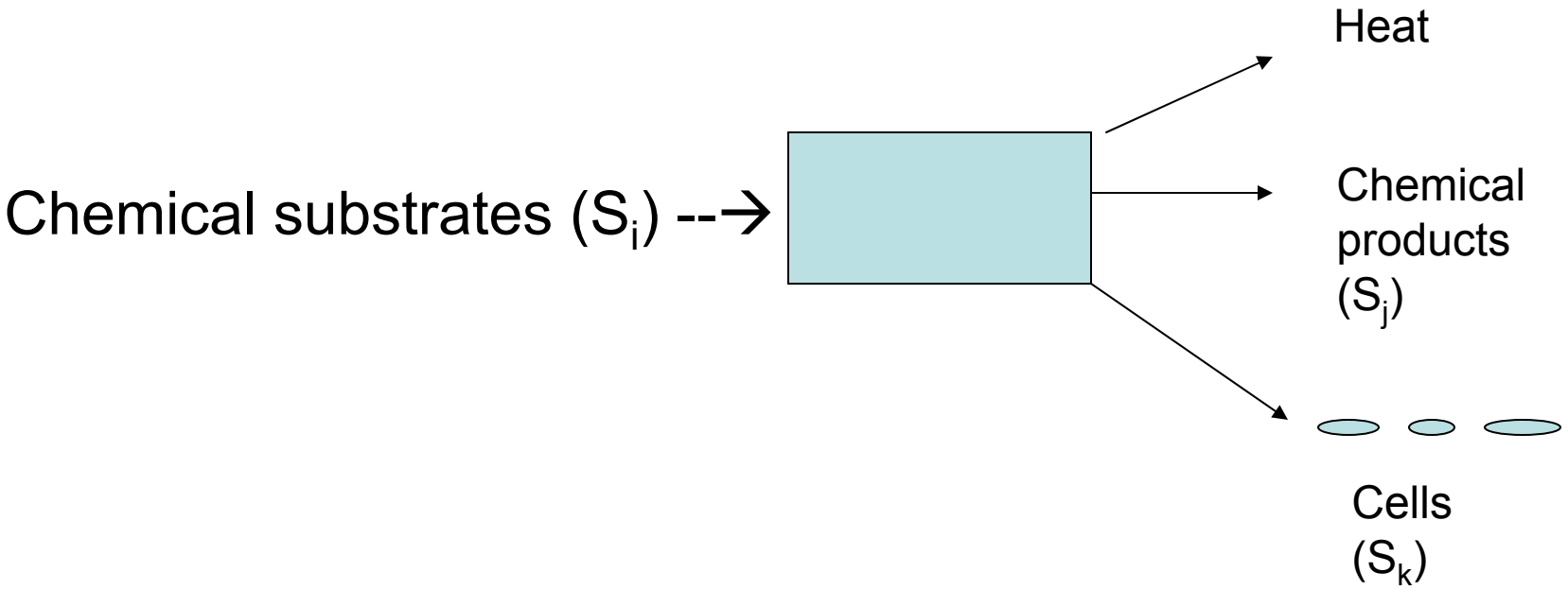
- Breads, yogurt, cheeses, etc…
- Recombinant DNA techniques are used to make cheese.
- Fermentation is a microbial process that is used to produce food products and beverages
- Fermentation: the oxidation of pyruvate (using electrons from NADH) to lactic acid (lactate fermentation) or ethanol (alcohol fermentation) – **oxygen is not present**.
  - NADH: nicotinamide adenine dinucleotide

Cell and Energy (Take home lessons)

- The energy consumed by the cells helps them to carry out reactions that occur inside the cells
- They use the energy to make products and reproduce
- They use the energy to transport nutrients
- They use the energy to change location
- Two main carriers of energy in living cells are: **A**denosine **T**ri**P**hosphate (ATP) and **N**icotinamide **A**denine **D**inucleotide (NAD+). They are high-energy carriers.

Stoichiometry and Energetics of growth

- The cell can be viewed as an open system:



Cell Cycle

- Events that occur during growth of a single cell, from inception till the time of its division into daughter cells are referred to as the “cell cycle”:

  – M phase: nuclear division (mitosis)
  – Interphase: daughter cells from the mitosis phase enters the G_1 phase
  – G_1 phase: a high rate of biosynthesis
  – S phase: DNA synthesis starts and ends with DNA of the cell has doubled.
  – G_2 phase: follows and ends with initiation of mitosis

  Te biochemical content of the cell is constantly changing over the cell cycle.

Cell kinetics

- It is important to understand the growth kinetics of microbial, animal or plant cells.
- This information can be used to design fermenters

Terminology

C_x: Cell concentration, dry cell weight per unit volume

C_n: Cell number density, number of cells per unit volume

ρ: Cell density, wet cell weights per unit volume of cell mass

dC_x/dt: Change of dry cell concentration with time

r_x: Growth rate of cells on a dry weight basis

dC_n/dt: Change of cell number density with time

r_n: Growth rate of cells on a number basis

δ: Division rate of cells on a number basis: $d\log_2 C_n/dt$.

Cell kinetics

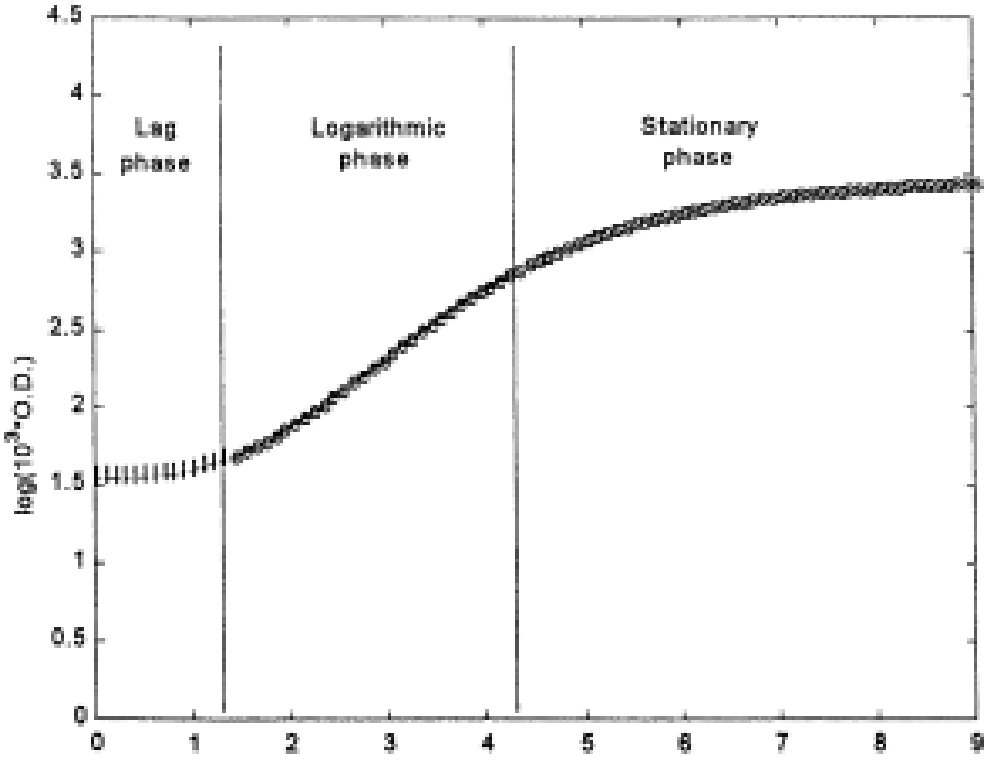
- $Cn=C_{no}\ 2^N$: All the cells in vessel at time t=0 ($Cn=C_{no}$) have divided once after a certain period of time. The cell population has increased to $C_{no}\times 2$. If the cells have divided N times we have $Cn=C_{no}\ 2^N$.
- The average division rate is: $\overline{\delta} = \frac{N}{t}$
- The average division rate can be written as: $\overline{\delta} = \frac{1}{t}\left(\log_2 C_n - \log_2 C_{n0}\right)$
- The division rate at time t is: $\delta = \frac{d \log_2 C_n}{dt}$

Growth Cycle for Batch Cultivation

When you inoculate unicellular microorganism in a fresh medium and measure the cell concentration with time:

- Lag phase: change of cell number is zero
- Accelerated growth phase: cell number starts to increase and division rate reach a maximum
- Exponential growth phase: cell number increases exponentially
- Decelerated growth phase: deceleration of both growth rate and division rate
- Stationary phase: cell population reaches a maximum value and stops to increase
- Death phase: nutrients for cells are depleted, cells starts to die and the number of viable cells decreases.

Growth Cycle for Batch Cultivation



Simon, L. and Karim, M.N. (2001), **Probabilistic Neural Networks using Bayesian decision strategies and a modified Gompertz model for growth phase classification in the batch culture of Bacillus subtilis**, *Biochemical Engineering Journal*, 7, 41-48.

Batch Cultivation – Exponential Growth Phase

- The rate of the cell population increase at any particular time is proportional to the number density of bacteria

$$r_n = \frac{dC_n}{dt} = \mu C_n$$

- μ is the specific growth rate [hr^{-1}]

- The specific growth rate is equal to ln(2) times the division rate

$$\mu = \frac{1}{C_n}\frac{dC_n}{dt} = \frac{d\ln\left(C_n\right)}{dt} = \ln\left(2\right)\times\frac{d\log_2\left(C_n\right)}{dt} = \delta\ln\left(2\right)$$

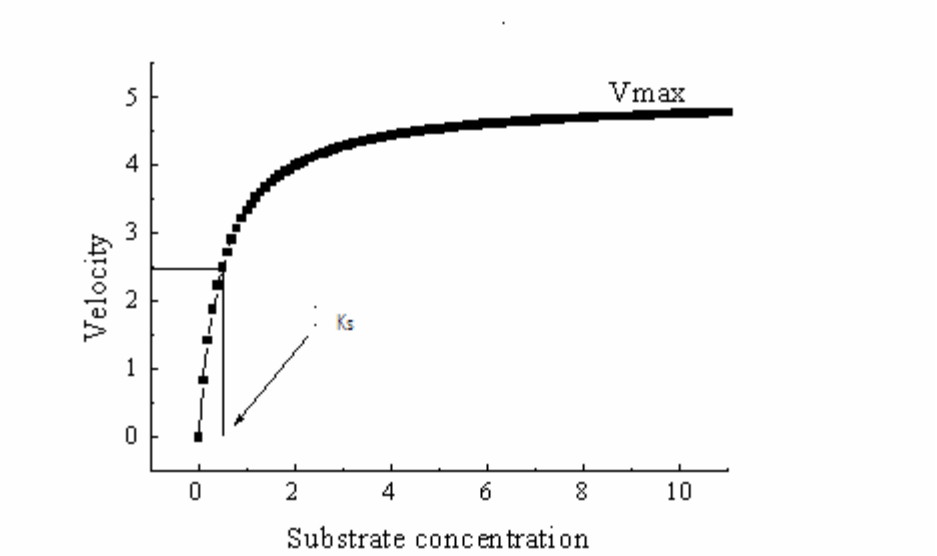
- The number cell concentration is:

$$C_n = C_{n0}\exp\left[\mu\left(t - t_o\right)\right]$$

- The doubling time: $t_d = \frac{\ln(2)}{\mu} = \frac{1}{\delta}$

Batch Cultivation – Factors Affecting the Specific Growth Rate

- Monod Equation $$\mu = \frac{\mu_{\max} C_s}{K_s + C_s}$$
  - Ks is the concentration of nutrient when the pecific growth rate is half its maximum value.
  - The equation is empirical
  - Experiments are usually carried out to estimate the model parameters



Substrate Kinetics

- The growth yield is defined as:

$$Y_{X/S} = \frac{\Delta C_X}{-\Delta C_S} = \frac{C_X - C_{X0}}{-\left(C_S - C_{S0}\right)}$$

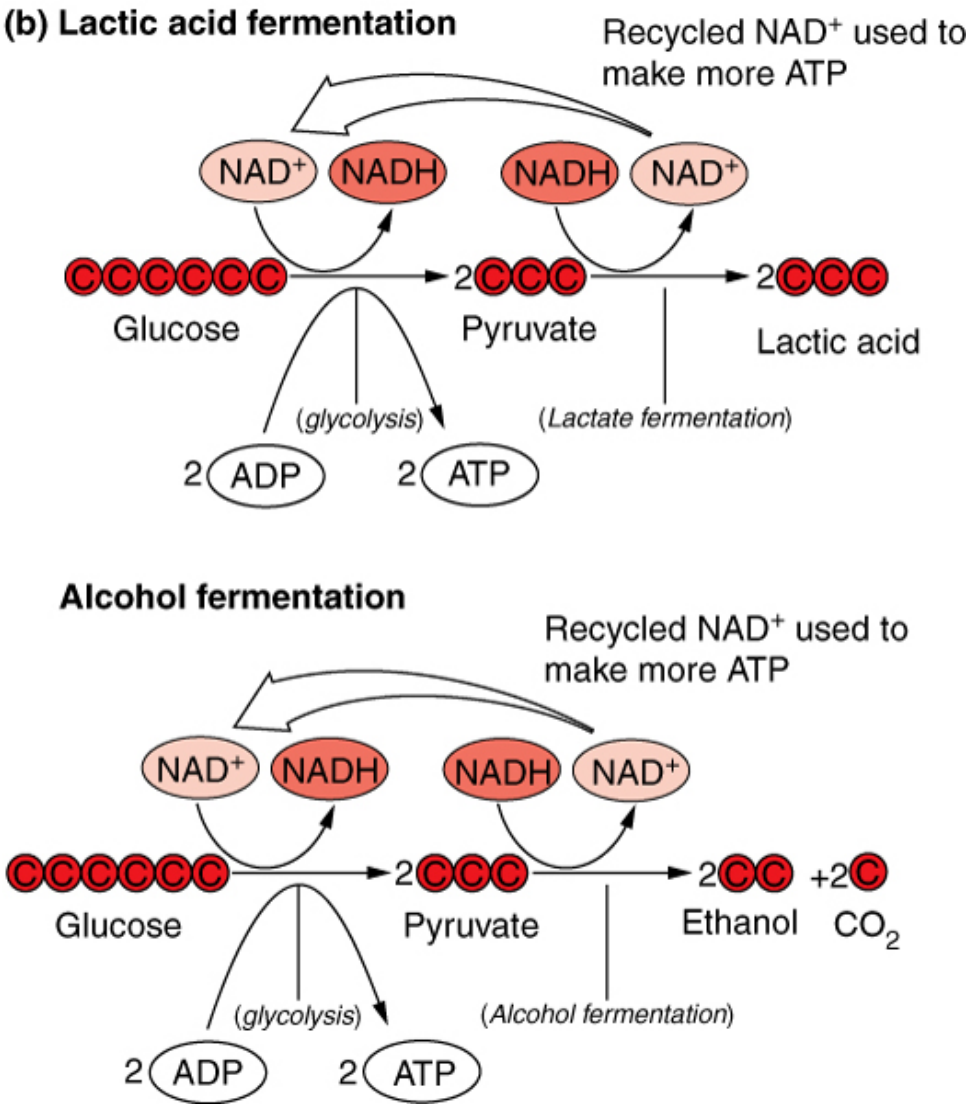
- Cell growth rate is related to substrate consumption by:

$$\frac{dC_X}{dt} = \mu C_X = -Y_{X/S}\frac{dC_S}{dt}$$

- Growth rate:

$$\frac{dC_X}{dt} = \frac{\mu_{\max} C_s C_X}{K_s + C_s}$$

Fermentation



Therapeutic Proteins

- Recombinant insulin from bacteria – insulin (from beta cells in the pancreas) helps to stimulate the uptake of glucose into body cells (ex: muscle cells).

- In the muscle cells, glucose is broken down to make ATP as an energy source

- **Type I** (insulin-dependent diabetes mellitus): inadequate production of insulin by beta cells -> elevated blood glucose concentration -> high blood pressure, poor circulation ect…

- Before recombinant DNA technology, source of insulin was: pancreases of pigs and cows

Microbes Against Other Microbes

- Antibiotics: can inhibit the growth of microbes
- Stop bacteria from replicating or kill the microbes

Case study:
The use of *Trichoderma reesei* to produce as β-glucosidase

- The enzyme plays a significant role in the paper, textile and food industries as well as in the production of ethanol.

- It is essential to increase the production of the enzyme and to devise reliable separation strategies.

Cultivation of *T. reesei*

- *T. reesei* strains were incubated on a potato dextrose agar (39 g/l) at 30 ◦C for 14 days.
- After incubation, 10% (v/v) inoculum was transferred to a shake flask, containing the cultivation medium
- Medium: 5 g (NH4)2·SO4, 15gKH2PO4, 0.3 g urea, 0.8 g CaCl2, 1.23 g MgSO4·7H2O, 0.0027 g FeSO4·7H2O, 0.0016 g MnSO4·H2O, 0.0014 g ZnSO4·7H2O, 0.75 g peptone, 10 g/l glucose, 0.5 g Tween 80, 0.3 g yeast extract and 10 g lactose in a liter of distilled water.

Cultivation of *T. reesei*

- The fermentation was carried out at 28 °C on a rotary shaker at 150 rpm for 4–5 days.

- Samples, collected every 24 h during the culture growth period, were centrifuged while the supernatant was analyzed for total protein concentration and -glucosidase activity

Cultivation of *T. reesei*

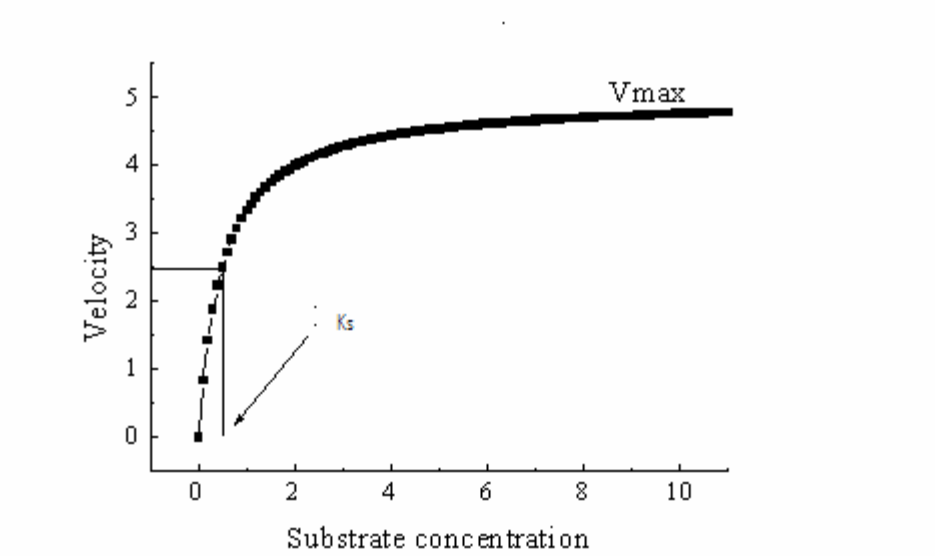
Cultivation of *T. reesei*

Batch Cultivation

- Inoculate the microorganism into a fresh sterilized medium.
- Main phases of growth (and death): lag, exponential, stationary and death phases.
- The specific growth rate will be affected by the substrate concentration (i.e., Monod equation)

Batch Cultivation – Factors Affecting the Specific Growth Rate

- Monod Equation $$\mu = \frac{\mu_{\max} C_s}{K_s + C_s}$$
  - Ks is the concentration of nutrient when the pecific growth rate is half its maximum value.
  - The equation is empirical
  - Experiments are usually carried out to estimate the model parameters

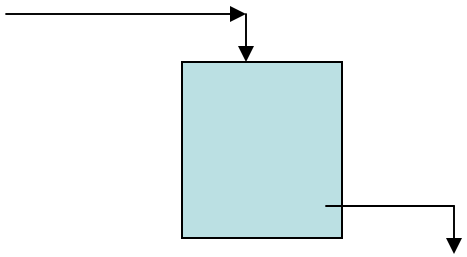


Ideal Continuous Stirred-tank Fermenter

- Drawbacks of batch systems: No fresh nutrient added; the cells die from starvation.

- Cells are maintained in a state of exponential growth over a long period of time by using a continuous system.

- **Inlet**: Flow: F; Concentration: C_{si}, C_{xi}, C_{pi}
- **Outlet**: Flow: F; Concentration: C_s, C_x, C_p
- **Vessel**: Volume: V; Concentration: C_s, C_x, C_p



Ideal Continuous Stirred-tank Fermenter (steady-state operation)

- Monod Kinetics: $\mu = \frac{\mu_{\max} C_s}{K_s + C_s}$

When $C_{xi} = 0$ and $r_x = \mu C_x$ (exponential growth)

Strategy: <u>fix μ</u>

- Dilution rate: $D = F/V = \mu$
- Residence time: $\tau = 1/\mu = 1/D = V/F$
- $C_s = (K_s)/[(\tau \mu_{max}) - 1]$
- $C_x = Y_{x/s} (C_{si} - C_s)$
- $C_p = C_{pi} + Y_{p/s} (C_{si} - C_s)$

Productivity of CSTF

- If the inlet stream is sterile, the productivity of cell mass is $C_x/\tau = DC_x$ (the amount of a cell mass produced per unit time and volume)

$$\frac{C_X}{\tau} = r_X = \frac{\mu_{\max} C_s C_X}{K_s + C_s}$$

Maximum Productivity of CSTF

- Optimum cell concentration:

$$C_{X,opt} = Y_{X/S} C_{Si} \frac{\alpha}{\alpha + 1} \qquad \alpha = \sqrt{\frac{K_S + C_{Si}}{K_S}}$$

- Optimum substrate concentration:

$$C_{S,opt} = \frac{C_{Si}}{\alpha + 1}$$

- Optimum residence time: $\tau_{opt} = \frac{\alpha}{\mu_{\max}(\alpha - 1)}$

- Optimum volume: $V_{opt} = \tau_{opt} F$

Physiologically Based Pharmacokinetics (PBPK) Models

- These models have been applied successfully to assess risk posed by toxic chemicals, and to elucidate their biological mechanisms as they are transported to specific organs and tissues.

- In the PBPK modeling framework, the body is divided into several physiological compartments, such as the liver and lung, and material balance equations are written to represent chemical uptake in the various tissues

PBPK Model

Material balance is used to model concentration profile of medications, poisons, etc.. in the body