Ch. 6 세포의 생장

- 생장: 복제와 세포 크기 변화의 결과
- 영양소
  - 에너지 생산
  - 생합성, 산물 제조

$\sum S + X \rightarrow \sum P + nX$: 자가촉매 (autocatalytic) 반응

- 비성장속도 (specific growth rate)

$\mu_{net}\ (h^{-1}) = (1/X)\ (dX/dt)$

$\mu_{net} = \mu_g - k_d$

알짜 비성장속도 = 전체 비성장속도 – 내인성 대사 또는 사멸에 의한 균체손실속도

Ch. 6 세포의 생장

- 알짜 비복제속도

$\mu_R\ (h^{-1}) = (1/N)\ (dN/dt)$

N: 세포수 농도

- 비기질 소모 속도

q_S (g 기질/g 바이오매스·h) $= -(1/X)\ (dS/dt) = -(1/X)\ (\Delta S/\Delta t)$

- 비생산 속도

q_P (g 생성물/g 바이오매스 ·h) $= (1/X)\ (예/dt) = (1/X)\ (\Delta P/\Delta t)$

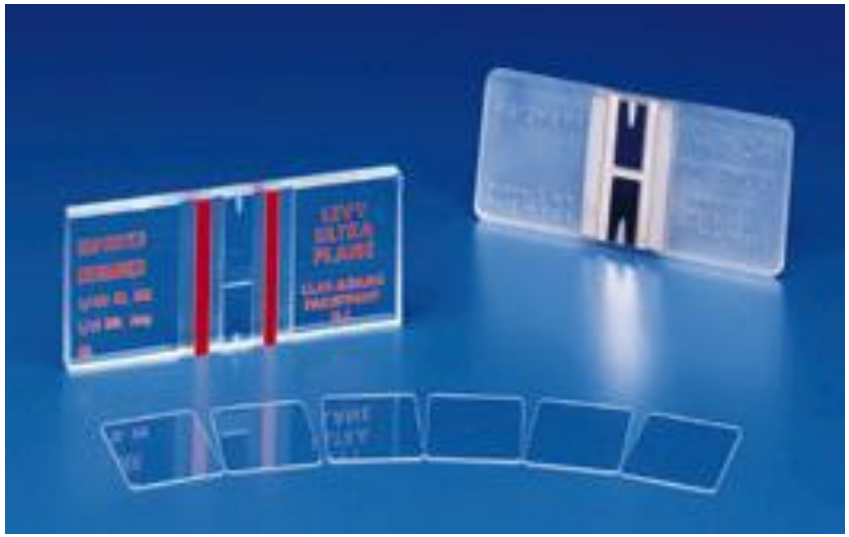
6.2 회분식 생장 (batch growth)

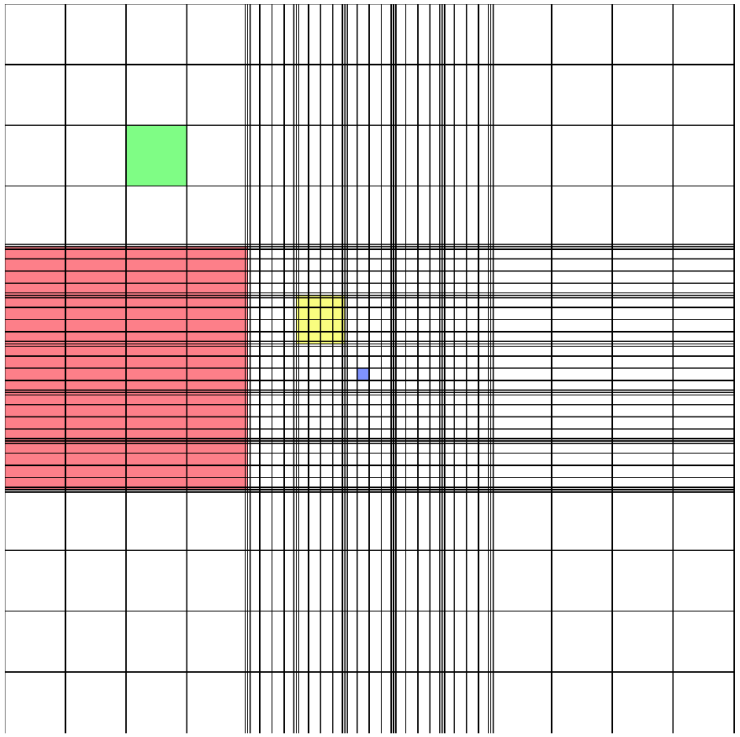
- 초기에 한번 배지를 채운 후 더 이상의 영양물질의 공급이나 제거가 없는 반응기에서 세포 배양
- 단순, 널리 사용
- 세포농도의 정량
  - 세포 수밀도 (number density)
  - 세포 질량농도 (mass concentration)

세포 수밀도의 결정

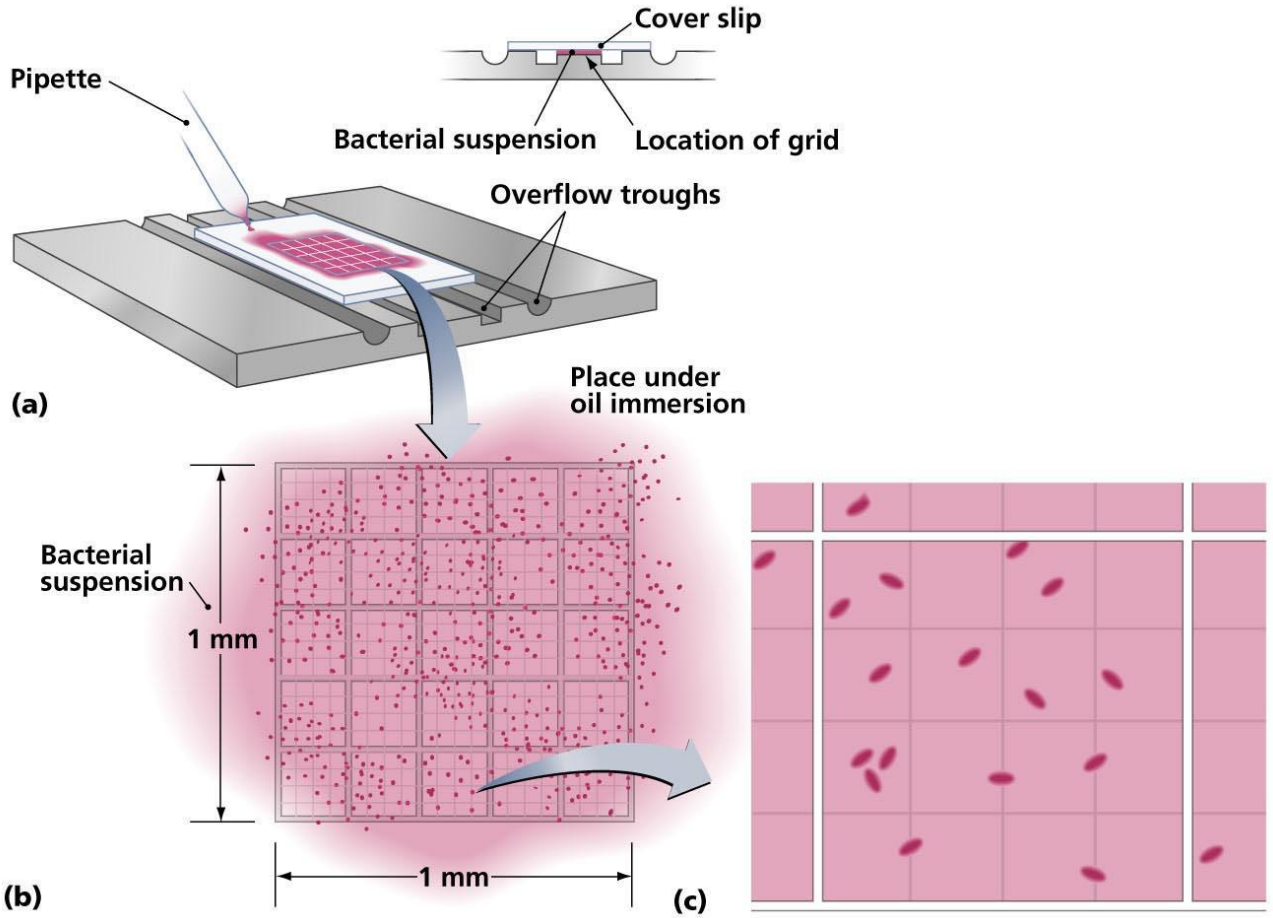
- Petroff-Hausser 슬라이드 (hemocytometer)
  - 간격이 일정하게 정해진 격자구조 (grid)를 culture chamber 위에 놓은 후 현미경을 통해 한칸 당의 세포숫자를 직접 셈
  - 적어도 20칸 이상을 세어 평균
  - 투명배지, 살아 있는 세포와 죽은 세포 구분: 염색
  - 세포가 응집되지 않는 배양계에 적합, 사상곰팡이 X
- 판 계수법 (plate counts)
  - 배지시료 희석, 한천 표면에 퍼뜨린 후 Petri dish 항온배양
    -> 군체형성단위 (CFU)
  - 사상균 X, 배지선택 주의
  - 눈에 보이는 군체 형성: 25세대 소요
- 입자 계수기: 전기저항 이용

Hemocytometer

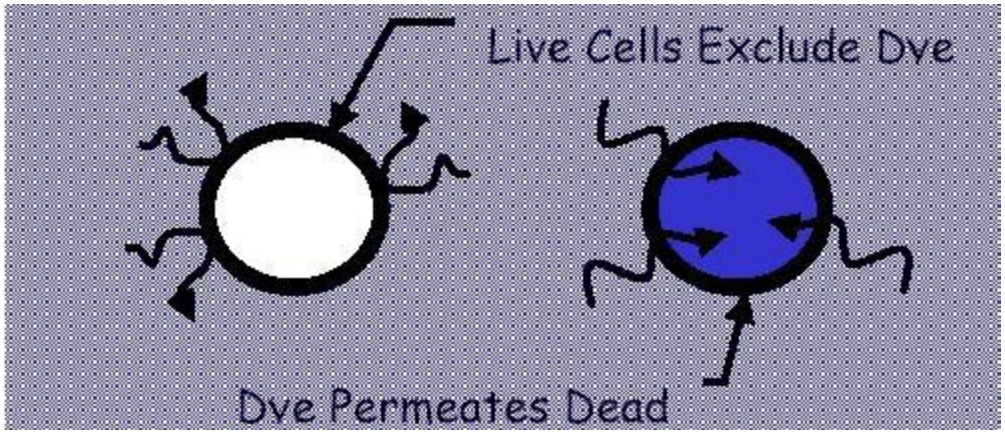




Hemocytometer



Trypan Blue Dye Exclusion Methods



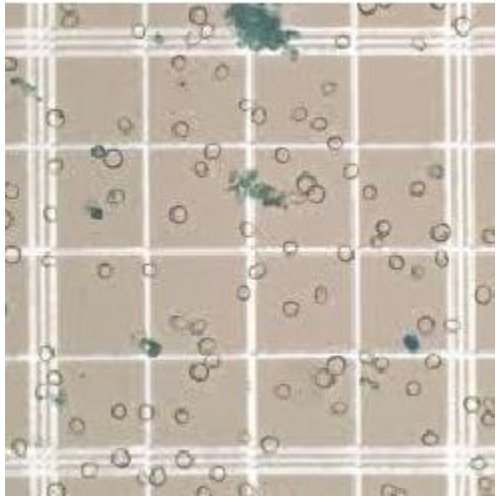
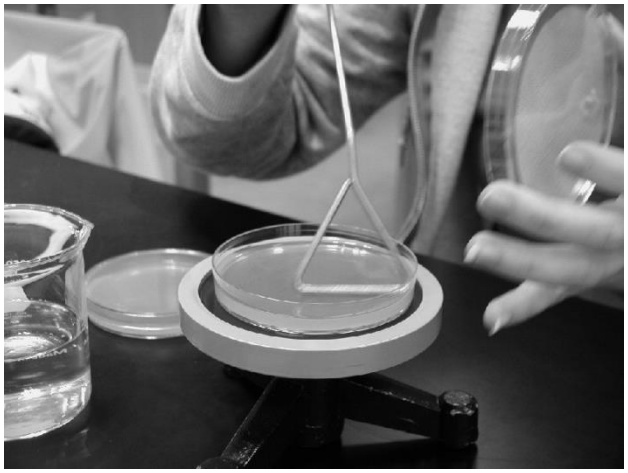
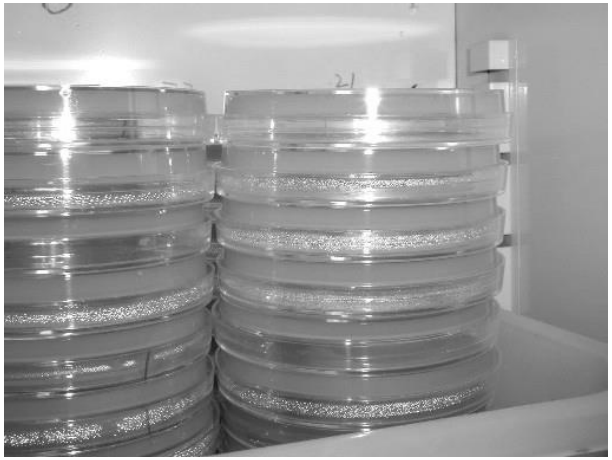


Plate Counts





Particle Counter

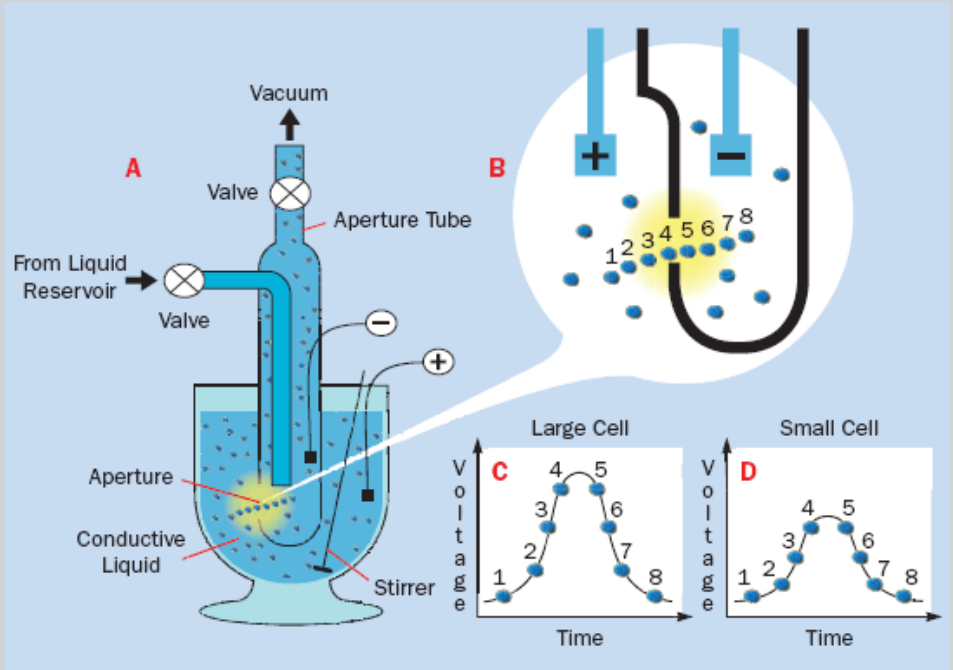


Figure 1. Simplified schematic of a Coulter Counter. As the vacuum pulls the suspension through the aperture in A, electric current flows between the electrodes. As individual particles travel through the sensitive zone (points 1-8) in B, voltage pulses are generated as shown in C and D. The large cell in C creates a larger amplitude pulse than the small cell in D since it causes greater electrical resistance through the aperture. The stirrer helps to keep the particles suspended, and liquid from the reservoir can be used to flush out the aperture tube between runs.

세포 질량농도의 결정: 직접법

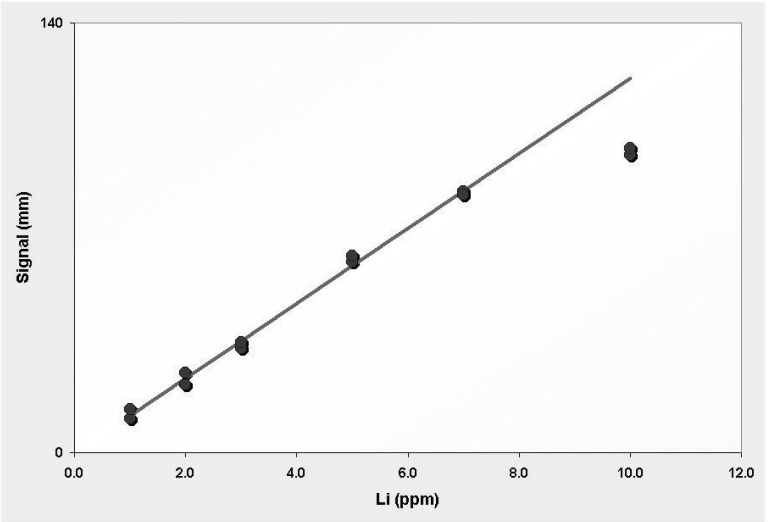
- 세포 건조중량
  - 고형물 (당밀, 셀룰로스, CSL) X
  - 시료 원심분리 or 여과, 완충용액 or 물로 세척, 80 °C, 24 h 건조
- 충전세포 부피 (packed cell volume)
  - 신속히 대충 추산
  - 배양액을 눈금이 매겨진 tapered tube에 넣어 표준 조건에서 원심분리후 세포가 차지하는 부피 측정
- 탁도 (turbidity) or 광학밀도 (optical density)
  - 분광계 (spectrophotometer) 이용
  - 세포가 빛을 흡수하는 정도
  - 배지성분에 의한 흡광을 최소화하는 파장 사용 (600 – 700 nm)
  - 보정 곡선: OD 0.3 이상에서 비선형성

Cell Mass (direct)

- Cell dry weight
  80~100 °C 24h in dry oven
- Packed cell volume
  centrifuge (rpm, time)

Cell Mass (direct)

Optical density (OD)
- Spectrophotometer
- 600 ~ 700 nm
- blanking (background)
- calibration (< 0.3)



세포 질량농도의 결정: 간접법

예) 사상곰팡이: 기질 소비량, 산물 생성량

- RNA, DNA, 단백질 측정
- 세포내 ATP 농도

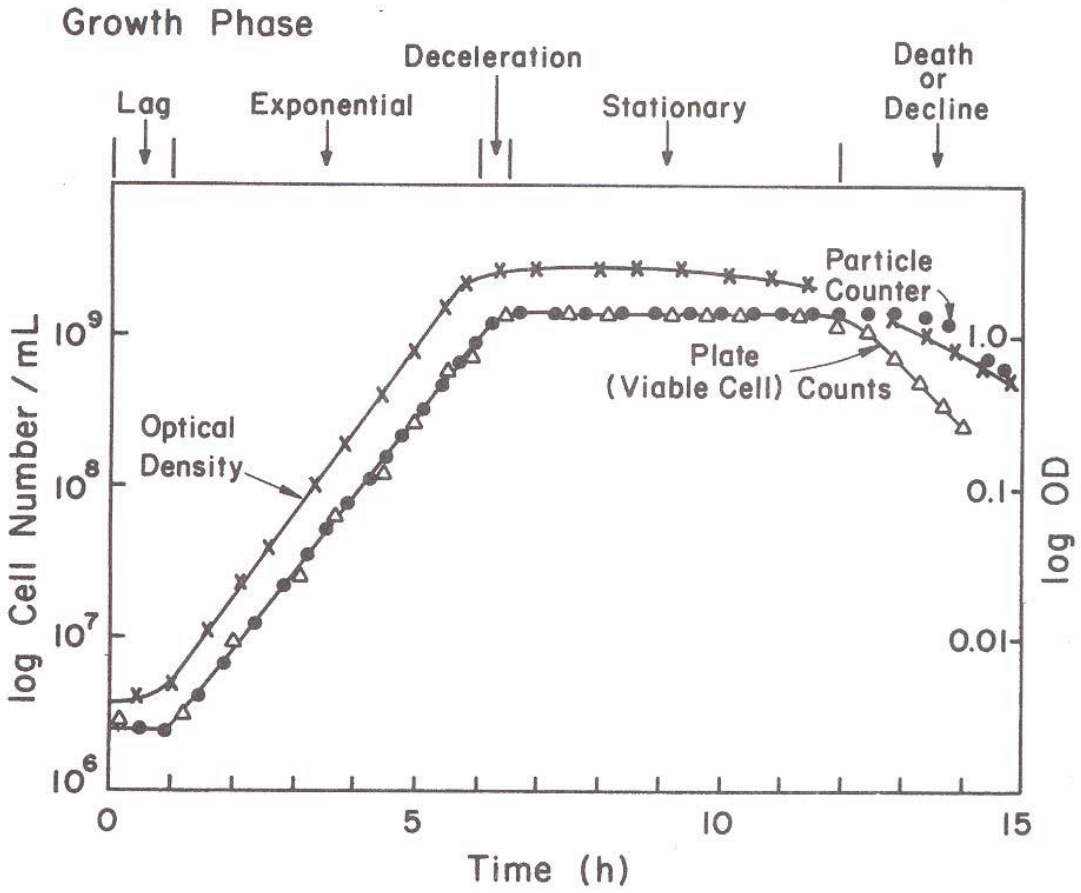
  luciferin + O_2 + ATP $\xrightarrow{\text{luciferase}}$ light

  – 총발광량 ∞ ATP 총량: 광도측정기 (photometer) or 섬광계수기 (scintillation counter) 이용
- 균체 생산에 쓰이는 영양물질 측정: 질산기, 인산기, 황산기, 탄소원 사용속도, 산소섭취속도
- 세포 대사산물: 에탄올, 젖산 (growth-associated)
- CO_2, pH 변화, pH 제어를 위한 산, 염기 공급속도
- 발효액 점도 변화

  – 균사체 생장, 다당류 생성: 점도 증가

  – 기질이 전분이나 셀룰로스: 점도 감소

Cell growth kinetics in batch culture



Lag phase

→ no increase in cell numbers

→ induction of enzymes to utilized substrate(s)

→ very important to decrease lag period to ↑ productivity

i. Inoculate with exponential phase cells

ii. Pre-acclimate inoculum in growth media

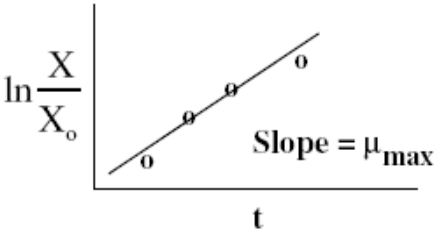
iii. Use high cell inoculum size (5-10% by volume)

Exponential growth phase

1. Nutrient and substrate concentrations are large
2. Growth rate is independent of nutrient and substrate conc.
3. Cell number and mass concentrations increase exponentially

$$\frac{dX}{dt} = \mu_{max} X,\ X = X_o \text{ at } t = 0$$

$$X = X_o e^{\mu_{max} t} \text{ or } \ln\frac{X}{X_o} = \mu_{max} t$$



doubling time of cells (t_d), $\frac{X}{X_o} = 2 \Rightarrow \ln(2) = \mu_{max} t_d$

$$t_d = \frac{\ln 2}{\mu_{max}} \quad \text{or} \quad \mu_{max} = \frac{\ln 2}{t_d}$$

4. Balanced growth occurs ⇒ cell composition constant

Deceleration phase

→ depletion of one or more nutrients

→ accumulation of toxic byproducts of growth

→ unbalanced growth and metabolism shifts for survival

Stationary phase

→ no net growth of cell numbers or cell mass (no cell division)

→ cell growth rate = cell death rate

→ *secondary metabolites* (products) produced

→ *endogenous metabolism* of energy stores can result in maintaining cell viability

→ removal of inhibitory compounds will result in further growth if additional substrate is provided

Death phase

1. Cell lysis (spillage) may occur
2. Rate of cell decline is first-order

$$\frac{dX}{dt} = -k_d' X \Rightarrow X = X_s \text{ at } t = 0$$

$$X = X_s e^{-k_d' t} \quad \text{or} \quad \ln\frac{X}{X_o} = -k_d' t$$

3. Growth can be re-established by transferring to fresh media

Effects of temperature on cell growth

μ_{max} doubles for each 10°C increase near T_{opt}

$$\mu_{max} = A\, e^{-E_a/RT} \quad \text{and}$$

$$k_d' = A' e^{-E_d/RT}$$

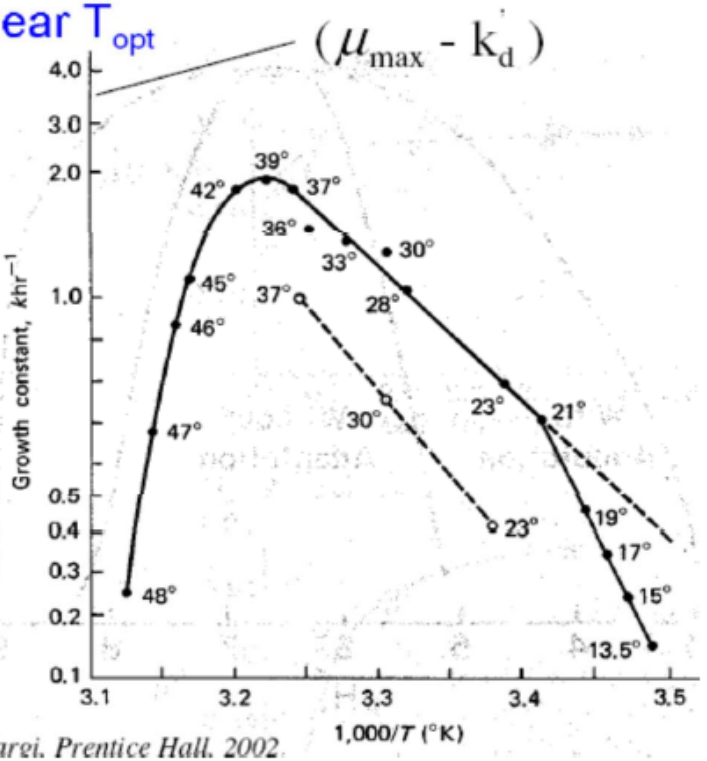
E_a = activation energy for growth

$\approx$ 10 to 20 kcal / mole

E_d = activation energy for death

$\approx$ 60 to 80 kcal / mole

As T ↑, k_d' ↑ faster than μ_{max}, $(\mu_{max} - k_d')$ ↓



"Bioprocess Engineering: Basic Concepts, Shuler and Kargi, Prentice Hall, 2002

pH effects

→ acceptable pH is ± 1 to 2 pH units

→ pH range varies by organism

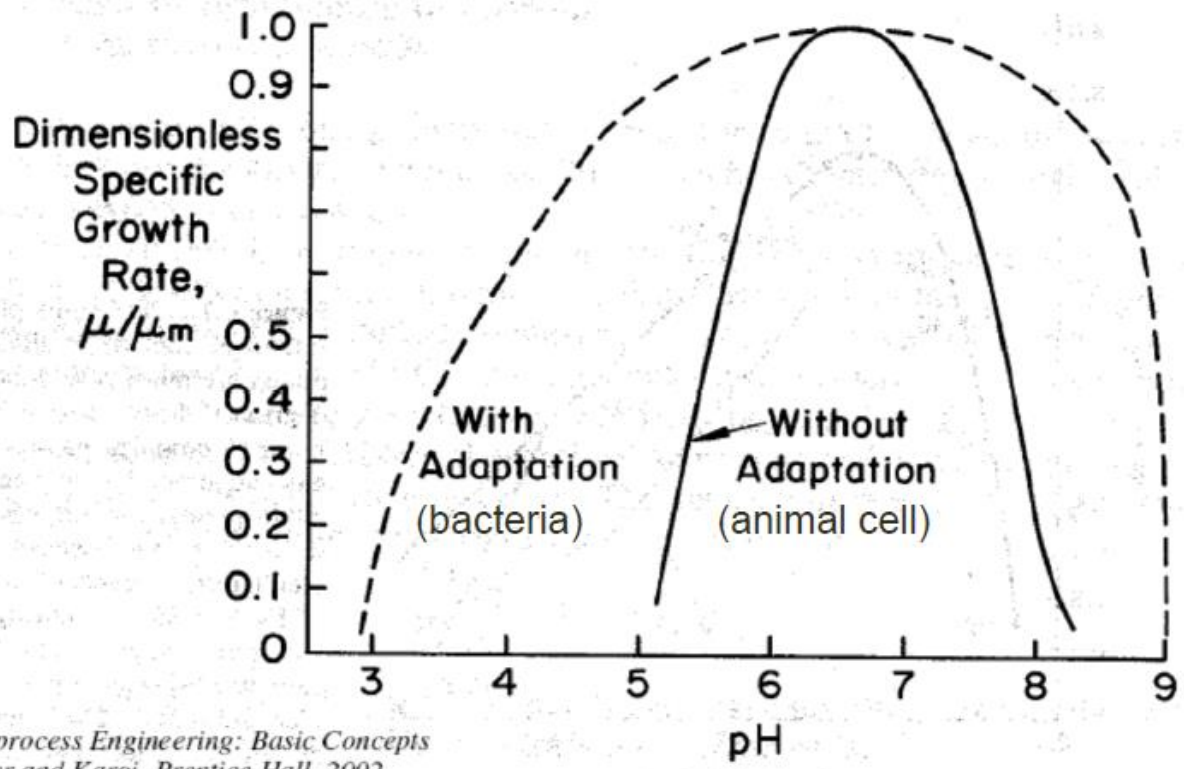
bacteria (most)	pH = 3 to 8
yeast	pH = 3 to 6
plants	pH = 5 to 6
animals	pH = 6.5 to 7.5

→ microorganism have the ability to control pH inside the cell, but this requires maintainance energy

→ pH can change due to

- utilization of substrates; NH_4^+ releases H^+, NO_3^- consumes H^+
- production of organic acids, amino acids, CO_2, bases

pH effects (cont.)



"Bioprocess Engineering: Basic Concepts
Shuler and Kargi, Prentice Hall, 2002

Effects of dissolved O_2 (DO)

→ O_2 may be a limiting substrate for aerobic fermentation, since O_2 is sparingly soluble in water

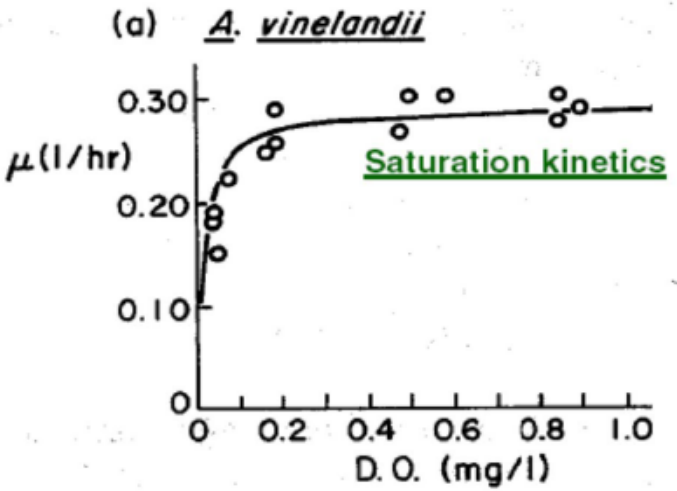
→ critical O_2 concentration

5 to 10% of saturation (≈ 7 mg O_2/L) for bacteria/yeast

→ growth exhibits saturation kinetics with respect to O_2 concentration (see next page)

Effects of dissolved O_2 (cont.)

Obligate aerobic cells



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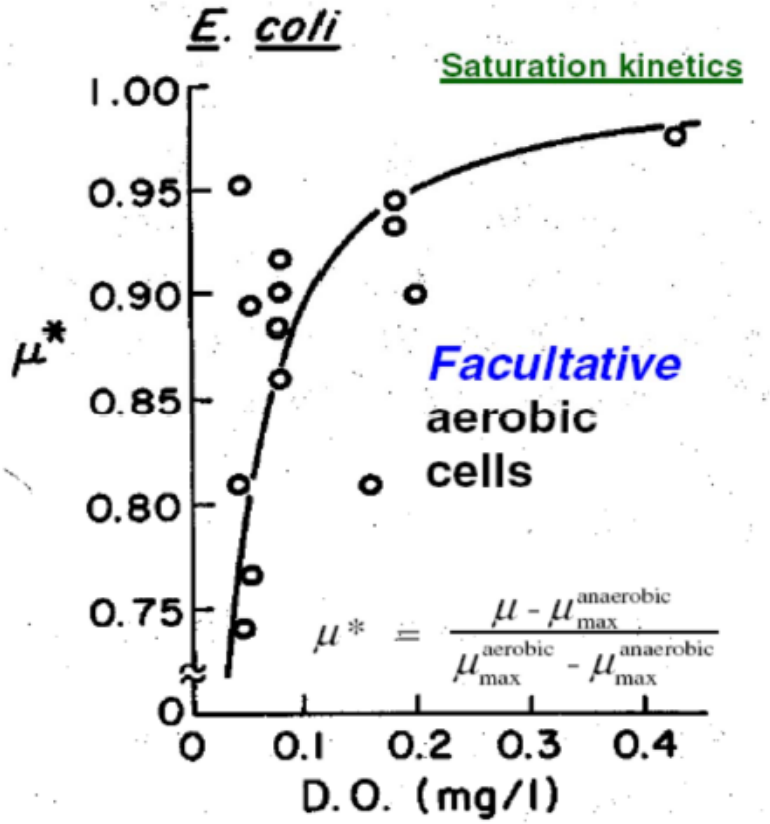

Other effects on cell growth

→ dissolved CO_2 (DCO_2); to high or low DCO_2 is toxic

→ ionic strength (I); too high dissolved salts is inhibitory to membrane function (membrane transport of nutrients, osmotic pressure)

$I = 1/2 \ \Sigma \ C_i Z_i^2$

C_i = molar concentration of ion i

Z_i = ion charge

→ maximum non-inhibitory concentrations of substrates, products

glucose (100 g/L), ethanol (10 g/L), NH_4^+ (5 g/L), ..

Growth Yield

- Apparent Yield, True Yield
- $\Delta S = \Delta S$ (Synthesis) + ΔS (Energy)

  = ΔS (Biomass) + ΔS (Extracellular product)
  + ΔS (Growth energy) + ΔS (Maintenance energy)

- $Y_{X/S} = -\Delta X/\Delta S$: aerobic (0.4~0.6)
- $Y_{X/O2} = -\Delta X/\Delta O_2$: aerobic (0.9~1.4)
- $Y_{P/S} = -\Delta P/\Delta S$

Yield Coefficients

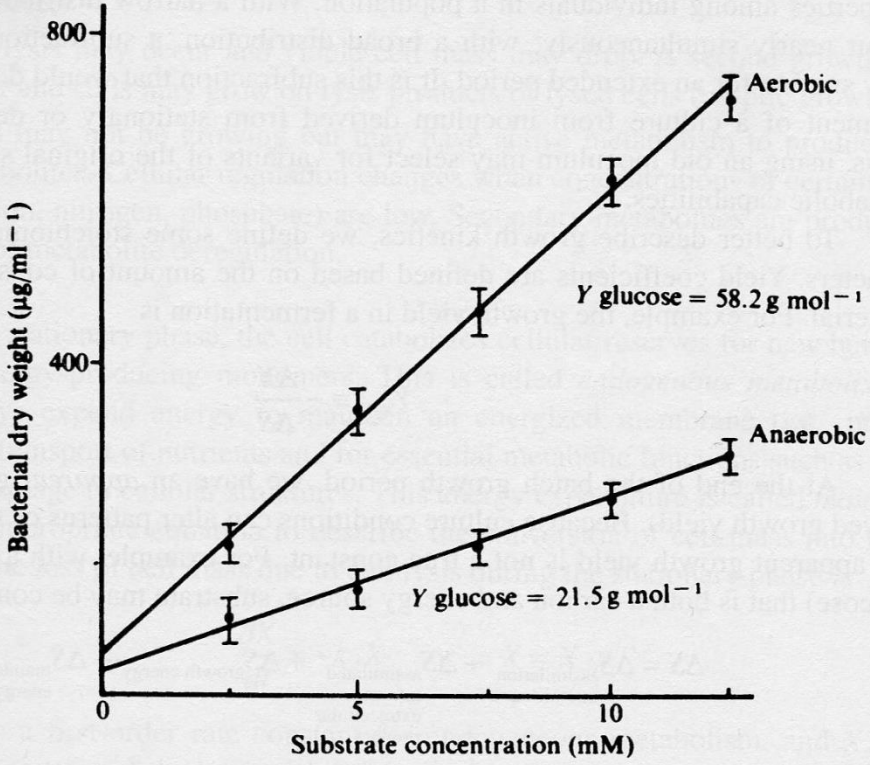

Figure 6.5. Aerobic and anaerobic growth yields of *Streptococcus faecalis* with glucose as substrate. (With permission, from B. Atkinson and F. Mavituna, *Biochemical Engineering and Biotechnology Handbook*, Macmillan, Inc., New York, 1983.)

Yield Coefficients

TABLE 6.1 Summary of Yield Factors for Aerobic Growth of Different Microorganisms on Various Carbon Sources

Organism	Substrate	$Y_{X/S}$ g/g	$Y_{X/S}$ g/mol	$Y_{X/S}$ g/g-C	Y_{X/O_2}[a] g/g
Enterobacter aerogenes	Maltose	0.46	149.2	1.03	1.50
	Mannitol	0.52	95.2	1.32	1.18
	Fructose	0.42	76.1	1.05	1.46
	Glucose	0.40	72.7	1.01	1.11
Candida utilis	Glucose	0.51	91.8	1.28	1.32
Penicillium chrysogenum	Glucose	0.43	77.4	1.08	1.35
Pseudomonas fluorescens	Glucose	0.38	68.4	0.95	0.85
Rhodopseudomonas spheroides	Glucose	0.45	81.0	1.12	1.46
Saccharomyces cerevisiae	Glucose	0.50	90.0	1.25	0.97
Enterobacter aerogenes	Ribose	0.35	53.2	0.88	0.98
	Succinate	0.25	29.7	0.62	0.62
	Glycerol	0.45	41.8	1.16	0.97
	Lactate	0.18	16.6	0.46	0.37
	Pyruvate	0.20	17.9	0.49	0.48
	Acetate	0.18	10.5	0.43	0.31
Candida utilis	Acetate	0.36	21.0	0.90	0.70
Pseudomonas fluorescens	Acetate	0.28	16.8	0.70	0.46
Candida utilis	Ethanol	0.68	31.2	1.30	0.61
Pseudomonas fluorescens	Ethanol	0.49	22.5	0.93	0.42
Klebsiella sp.	Methanol	0.38	12.2	1.01	0.56
Methylomonas sp.	Methanol	0.48	15.4	1.28	0.53
Pseudomonas sp.	Methanol	0.41	13.1	1.09	0.44
Methylococcus sp.	Methane	1.01	16.2	1.34	0.29
Pseudomonas sp.	Methane	0.80	12.8	1.06	0.20
Pseudomonas sp.	Methane	0.60	9.6	0.80	0.19
Pseudomonas methanica	Methane	0.56	9.0	0.75	0.17

[a] Y_{X/O_2} is the yield factor relating grams of cells formed per gram of O_2 consumed.

With permission, from S. Nagai in *Advances in Biochemical Engineering*, vol. 11, T. K. Ghose, A. Fiechter, and N. Blakebrough, eds., Springer-Verlag, New York, p. 53, 1979.

Yield Coefficients

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Methylococcus sp.	Methane	1.01	16.2	1.34	0.29
Pseudomonas sp.	Methane	0.80	12.8	1.06	0.20
Pseudomonas sp.	Methane	0.60	9.6	0.80	0.19
Pseudomonas methanica	Methane	0.56	9.0	0.75	0.17

[a] Y_{X/O_2} is the yield factor relating grams of cells formed per gram of O_2 consumed.

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Product yield coefficients

1. Growth associated products: products appear simultaneously with cells in culture

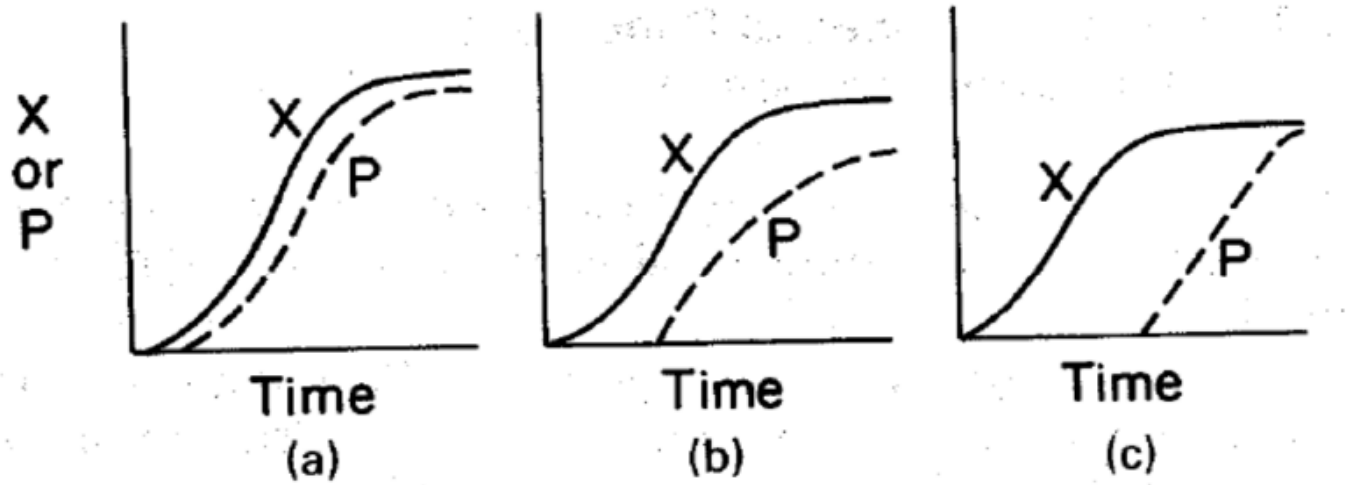
$$q_P = \frac{1}{X}\frac{dP}{dt} = Y_{P/X}\,\mu$$

2. Non-growth associated products: products appear during stationary phase of batch growth

$$q_P = \beta$$

3. Mixed-growth associated products: products appear during slow growth and stationary phase $q_P = \alpha\mu + \beta$

Product yield coefficients (cont.)



$$1.\ q_P = \frac{1}{X}\frac{dP}{dt} = Y_{P/X}\,\mu \qquad 3.\ q_P = \alpha\mu + \beta \qquad 2.\ q_P = \beta$$

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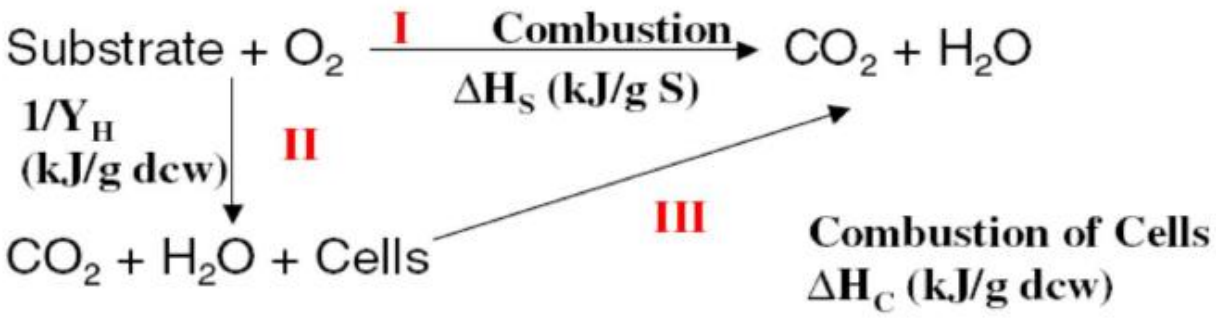
Heat generation by growth

Only 40 to 50% of the energy stored in a carbon substrate is converted to biological energy (ATP) during aerobic metabolism. The remainder is released as heat upon conversion to CO_2 and H_2O

Energy Balance: $\left[\begin{matrix}\text{Total Available}\\ \text{Energy of Substrate}\end{matrix}\right]^{I} = \left[\begin{matrix}\text{Energy Released}\\ \text{by Growth}\end{matrix}\right]^{II} + \left[\begin{matrix}\text{Energy Available}\\ \text{in Biomass}\end{matrix}\right]^{III}$



Heat generation by growth (cont.)

On a per gram of substrate basis

$$(1\ g\ S)\ \Delta H_S = (1\ g\ S)\ Y_{X/S}/Y_H + (1\ g\ S)\ Y_{X/S}\ \Delta H_C$$

Cell growth — Cell combustion

Solving for Y_H [cell growth per unit metabolic heat (g/cal)]

$$Y_H = \frac{Y_{X/S}}{(\Delta H_S - Y_{X/S}\ \Delta H_C)}$$

Typical ΔH_C = 20 to 25 kJ/g dcw

$1/Y_H$; metabolic heat per gram cell produced

Heat generation by growth (cont.)

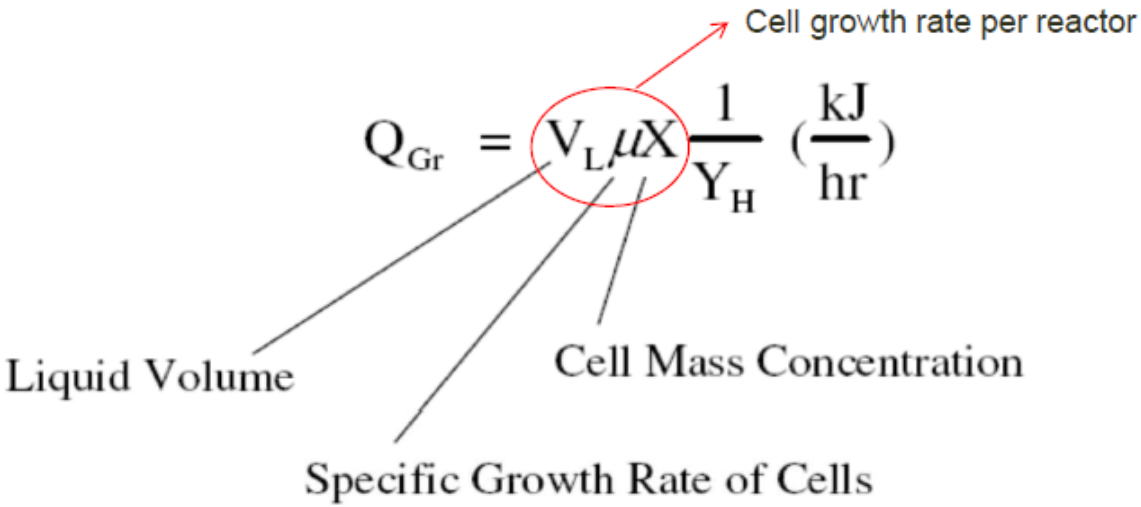
For Substrates:

S	ΔH_S (kJ/g S)	Y_H (g dcw/kJ)
Glucose	15.64	0.072
Methanol	22.68	0.029
Ethanol	29.67	0.043
n-Decane	47.64	0.038
Methane	55.51	0.015

The oxidation state of S has a large effect on 1/ Y_H

Rate of heat generation by growth, Q_{Gr}

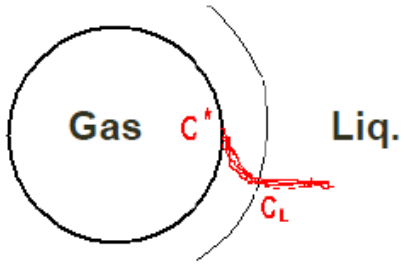
How can cell growth rate be correlated to heat generation?



Heat can be removed by circulating cooling water through an external jacket around the reactor vessel or through a coiled tube within the reactor.

Oxygen transfer rate (OTR)

- **Oxygen transfer from gas to liquid**



- **OTR = $k_L a (C^* - C_L)$**
  - OTR [mg O_2 / L / h]
  - k_L : oxygen transfer coefficient (cm/h)
  - a : gas-liquid interfacial area per unit vol. (cm^2/cm^3)
  - k_L a : volumetric oxygen transfer coefficient (1/h)
  - C* : saturated DO concentration (mg/L)
  - C_L : DO concentration in the broth (mg/L)

Oxygen uptake rate (OUR)

OUR from liquid to cell

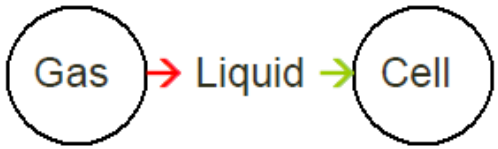
$$OUR = q_{O2} X = (\mu X) / Y_{X/O2}$$

- OUR [mg O_2 / L / h]
- q_{O2} : specific rate of oxygen consumption (mg O_2/g cell/h)
- $Y_{X/O2}$: oxygen yield coefficient (g cell/g O_2)

When oxygen transfer is the rate-limiting step,

$$OTR (\rightarrow) = OUR (\rightarrow)$$

$$k_L a (C^* - C_L) = (\mu X) / Y_{X/O2}$$



$$Y_{X/O2} k_L a (C^* - C_L) = dX / dt$$

$$Y_{X/O2} \cdot OTR = \text{cell growth rate}$$

Oxygen uptake rate (OUR)

OUR from liquid to cell

$$OUR = q_{O2} X = (\mu X) / Y_{X/O2}$$

- OUR [mg O_2 / L / h]
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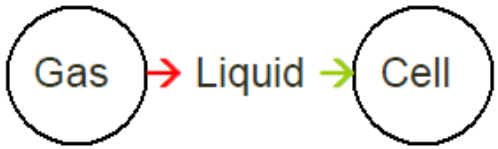


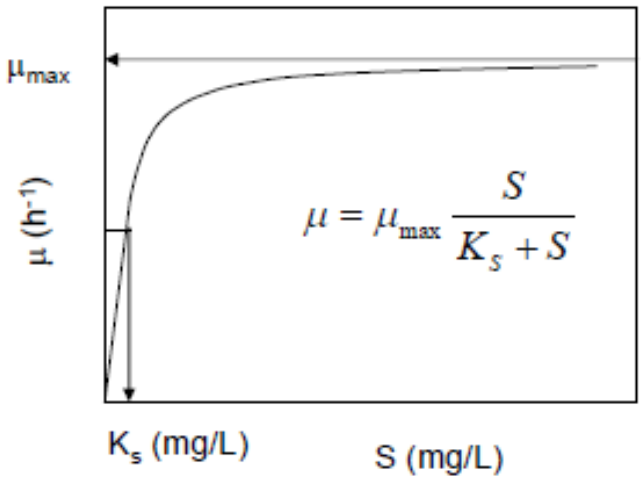
$$Y_{X/O2} k_L a (C^* - C_L) = dX / dt$$

$$Y_{X/O2} \cdot OTR = \text{cell growth rate}$$

모델의 관점들

Various Models For Cell Kinetics		Cell Components	
		Unstructured	Structured
Cell Population	Distributed	Cells are represented by a single component, which is uniformly distributed throughout the culture.	Multiple cell components, uniformly distributed throughout the culture interact with each others.
	Segregated	Cells are represented by a single component, but they form a heterogeneous mixture.	Cells are composed of multiple components and form a heterogeneous mixture.

Monod Equation



Rate equations (X_g, X_d, S_u)

$$r_g = \frac{\mu_m S}{K_s + S} X$$

$$r_d = k_d X$$

$$r_s = -\frac{q_m S}{K_s + S} X$$

$$\mu_m = Y_{X/S} q_m$$

Mass balance (X, S)

$$\frac{dX}{dt} = r_g - r_d$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}} r_g$$

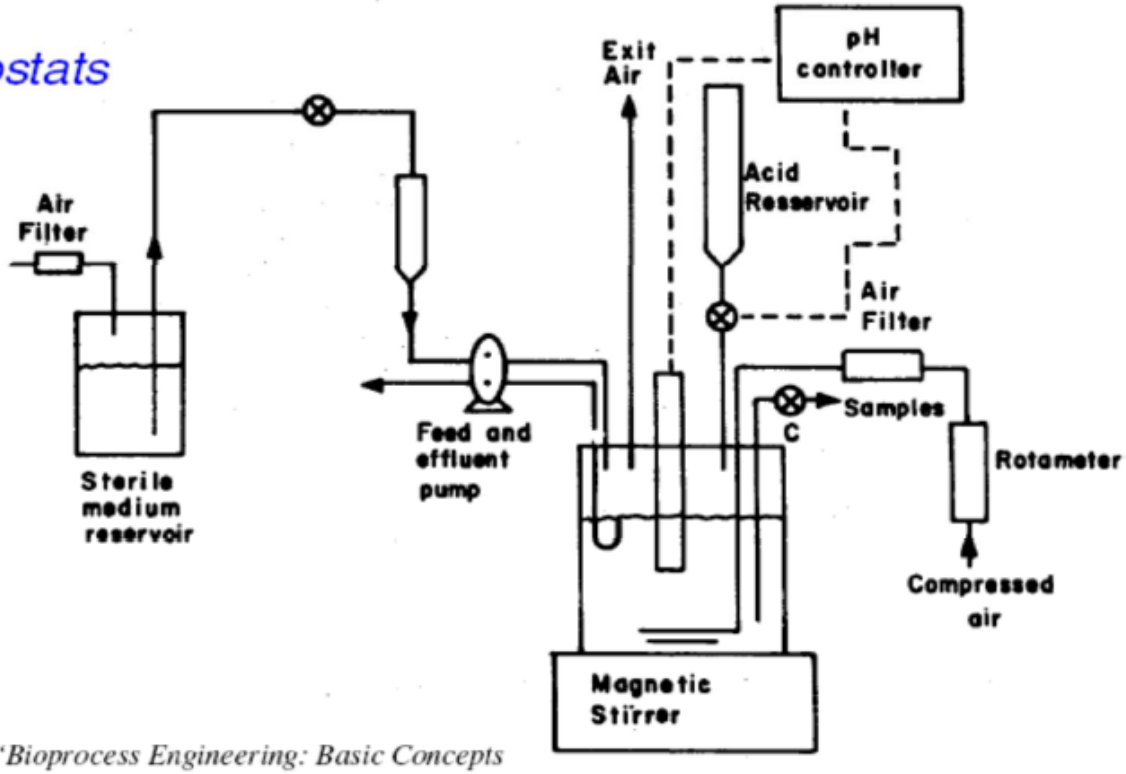
⇨ Different μ : Table 6.2

Cell growth in continuous culture

Automated Chemostats

→ control of pH, temp. agitation, dissolved oxygen

→ sterilization required



"Bioprocess Engineering: Basic Concepts
Shuler and Kargi, Prentice Hall, 2002

Chemostat as a tool

→ evaluate K_S, μ_{max}, $Y_{X/S}$ and other system parameters

→ study changes in environment and effects on cell physiology

→ select for cells with desired metabolic capabilities (e.g. selection for cells capable of degrading a toxic compound)

Chemostat mass balance

Why derive mass balance equation?

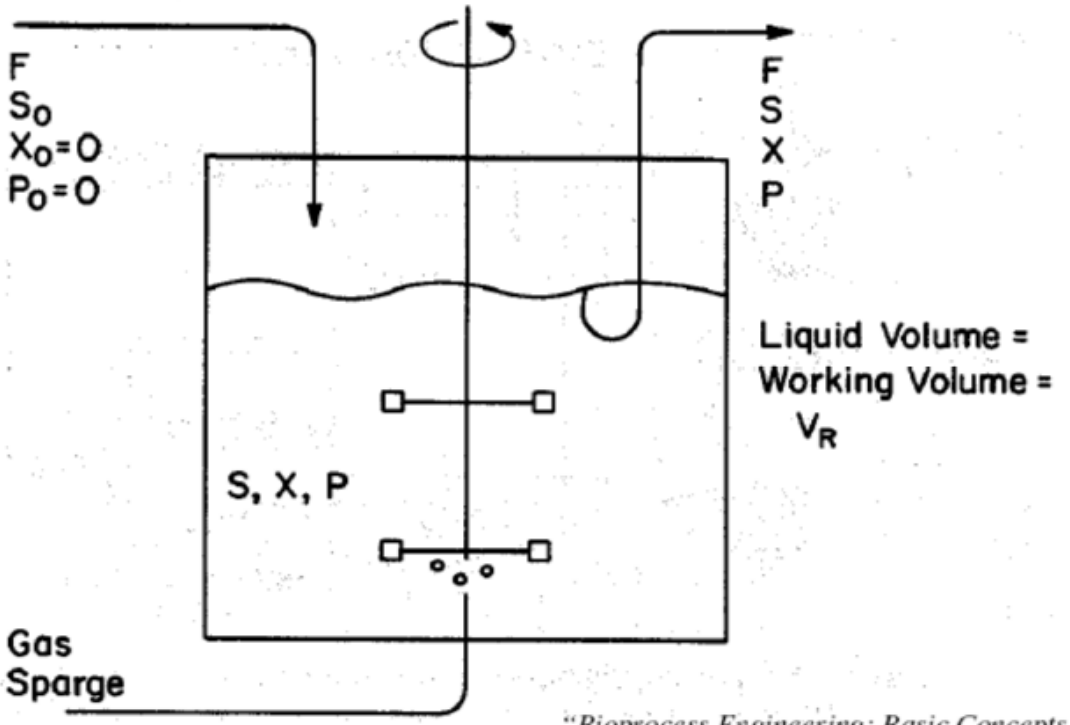
1. Describe dynamics of cell growth, substrate utilization, and product formation.

2. Useful for control of bioreactors.

3. Evaluate kinetic and yield parameters. ($Y_{x/s}$, K_s, μ_{max})

4. Determine the optimum values for bioreactor operating parameters.

Continuous-stirred tank reactor (CSTR), chemostat



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Shuler and Kargi, Prentice Hall, 2002

Cell mass balance in CSTR to get S

$$\begin{bmatrix}\text{mass rate}\\ \text{of cells into}\\ \text{bioreactor}\end{bmatrix} - \begin{bmatrix}\text{mass rate}\\ \text{of cells out}\\ \text{of bioreactor}\end{bmatrix} + \begin{bmatrix}\text{mass rate of cell}\\ \text{growth without}\\ \text{endogenous}\\ \text{metabolism}\end{bmatrix} - \begin{bmatrix}\text{mass rate}\\ \text{of cell loss}\\ \text{by endogenous}\\ \text{metabolism}\end{bmatrix} = \begin{bmatrix}\text{mass rate}\\ \text{of cells}\\ \text{accumulation}\\ \text{in bioreactor}\end{bmatrix}$$

or

$$FX_o - FX + V_R\mu X - V_R k_d X = V_R \frac{dX}{dt}$$

F = in and out volumetric flow rate (L/hr)

X = bioreactor and outlet cell mass concentration (g/L)

X_o = inlet cell mass concentration (g/L) = 0

μ = specific cell growth rate neglecting endogenous metabolism (hr^{-1})

k_d = endogenous cell loss rate constant (hr^{-1})

Steady state and sterile feed

Chemostats are normally operated at steady-state, d/X/dt = 0. Assume a sterile feed ($X_o = 0$), and k_d is so small that is neglected, $k_d = 0$.

The cell mass balance equations becomes,

$$\begin{bmatrix}\text{mass rate}\\ \text{of cells out}\\ \text{of bioreactor}\end{bmatrix} = \begin{bmatrix}\text{mass rate of cell}\\ \text{growth without}\\ \text{endogenous}\\ \text{metabolism}\end{bmatrix}$$

or

$$FX = V_R \mu X$$

$$\frac{F}{V_R} = \mu \quad \text{or} \quad \boxed{D = \mu}$$

where $\frac{F}{V_R} = D$, dilution rate

D [sec^{-1}] ; how many times of rxtor vol. flow per second

Substrate concentration in CSTR when $k_d = 0$

Using the Monod Equation, we can predict the bioreactor and outlet stream concentration of Substrate.

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

rearranging, $$\boxed{S = \frac{K_S D}{\mu_{max} - D}}$$

Substrate concentration in CSTR when $k_d \neq 0$

From cell mass balance

$$F X = V_R \mu X - V_R k_d X$$

$$F = V_R (\mu - k_d)$$

$$D = \mu - k_d$$

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

$$S = \frac{K_s (D + k_d)}{\mu_{max} - D - k_d}$$

→ S is higher than the case when $k_d = 0$

Substrate mass balance in CSTR to get X

How is X affected by D? A similar mass balance equation for S *in the absence* of endogenous metabolism is written to answer this question.

$$FS_o - FS - V_R \mu X \frac{1}{Y^M_{X/S}} - V_R q_p X \frac{1}{Y_{P/S}} = V_R \frac{dS}{dt}$$

S = bioreactor and outlet substrate concentration (g/L)

S_o = inlet substrate concentration (g/L)

$Y^M_{X/S}$ = maximum cell yield coefficient (g cells/g substrate)

$Y_{P/S}$ = product yield coefficient (g product/g substrate)

q_p = specific rate of extracellular product formation $\left(\frac{\text{g P}}{\text{g cells} \bullet \text{hr}}\right)$

Cell concentration in CSTR

For the simple case of no product formation ($q_p=0$), steady-state ($dS/dt=0$), and no endogenous metabolism, $k_d=0$.

$$D(S_o - S) = \frac{\mu X}{Y^M_{X/S}}$$

at steady-state, $\mu = D$, and solving for X,

$$X = Y^M_{X/S}(S_o - S)$$

or

$$X = Y^M_{X/S}\left(S_o - \frac{K_S D}{\mu_{max} - D}\right)$$

when $k_d \neq 0$

Thus far, the substrate balance eqn. Has been written assuming that $Y_{X/S}$ is a constant at $Y^M_{X/S}$.

With endogenous metabolism,

$\mu = D + k_d$

and with no extracellular product formation, the substrate mass balance is at steady-state,

$$D\frac{(S_o - S)}{X} - \frac{(D+k_d)}{Y^M_{X/S}} = 0$$

rearranging,

$$D\frac{(S_o - S)}{X} - \frac{D}{Y^M_{X/S}} - \frac{k_d}{Y^M_{X/S}} = 0$$

and

$$\frac{D}{Y^{AP}_{X/S}} - \frac{D}{Y^M_{X/S}} - \frac{k_d}{Y^M_{X/S}} = 0$$

$$\frac{1}{Y^{AP}_{X/S}} = \frac{1}{Y^M_{X/S}} + \frac{m_S}{D} = 0$$

where $m_S = \frac{k_d}{Y^M_{X/S}}$

maintenance coefficient based on S.

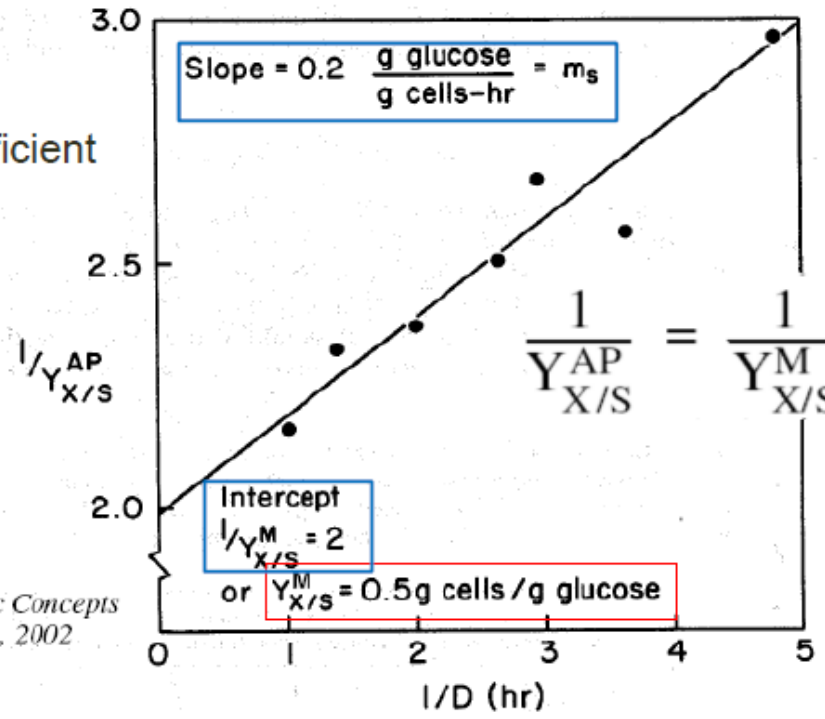
Measurement of maximum cell yield and maintenance using a chemostat

From measurements of X, S, S_o, and D in a chemostat experiment at different D values, a double reciprocal plot can be made.

Maintenance coefficient

$$\text{Slope} ; m_S = \frac{k_d}{Y^M_{X/S}}$$

$$k_d = m_S Y^M_{X/S}$$



$$\frac{1}{Y^{AP}_{X/S}} = \frac{1}{Y^M_{X/S}} + \frac{m_S}{D} = 0$$

"Bioprocess Engineering: Basic Concepts Shuler and Kargi, Prentice Hall, 2002

Determination of μ_{max} and K_s using a chemostat

From data collected using a chemostat, we can obtain the Monod Equation kinetic parameters.

Data include S at several Dilution Rates (D),

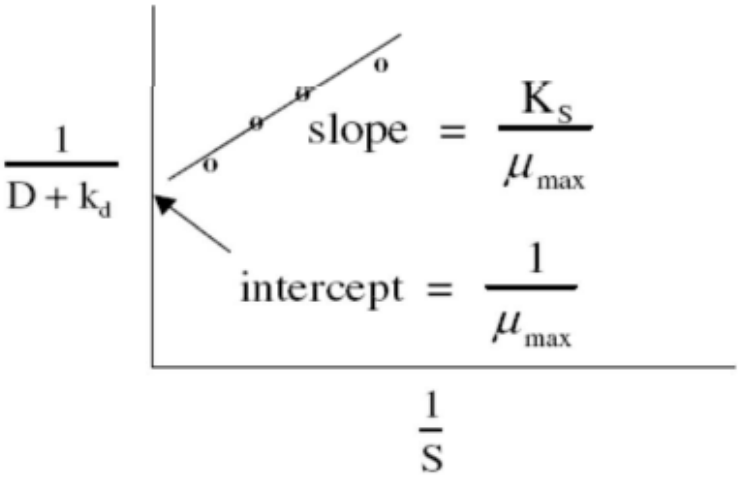
Recall that,

$$D = \mu - k_d \quad \text{(when } k_d \neq 0\text{)}$$

$$D = \frac{\mu_{max} S}{K_S + S} - k_d$$

rearranging

$$\frac{1}{D + k_d} = \frac{1}{\mu_{max}} + \frac{K_S}{\mu_{max}} \frac{1}{S}$$



Productivity of a chemostat

Cell production rate in CSTR [g/h] = FX

Pr_X = productivity for cell production = DX = FX / V

Pr_P = productivity for product formation = DP

The dilution rate (D) which maximizes productivity is found by taking dPr/dD = 0 and solving for D ($D_{optimum}$).

For example, $D_{optimum}$ for X with $k_d = 0$ and $q_P = 0$

$$X = Y^{M}_{X/S}(S_o - \frac{K_S D}{\mu_{max} - D}) \quad \Rightarrow \quad DX = Y^{M}_{X/S} D(S_o - \frac{K_S D}{\mu_{max} - D})$$

take $\frac{d(DX)}{dD} = 0$ and solve for D (D_{opt})

$$D_{opt} = \mu_{max}(1 - \sqrt{\frac{K_S}{K_S + S_o}})$$

K_S is usually << S
so D_{opt} ~ μ_{max} (washout point)

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K_S is usually << S
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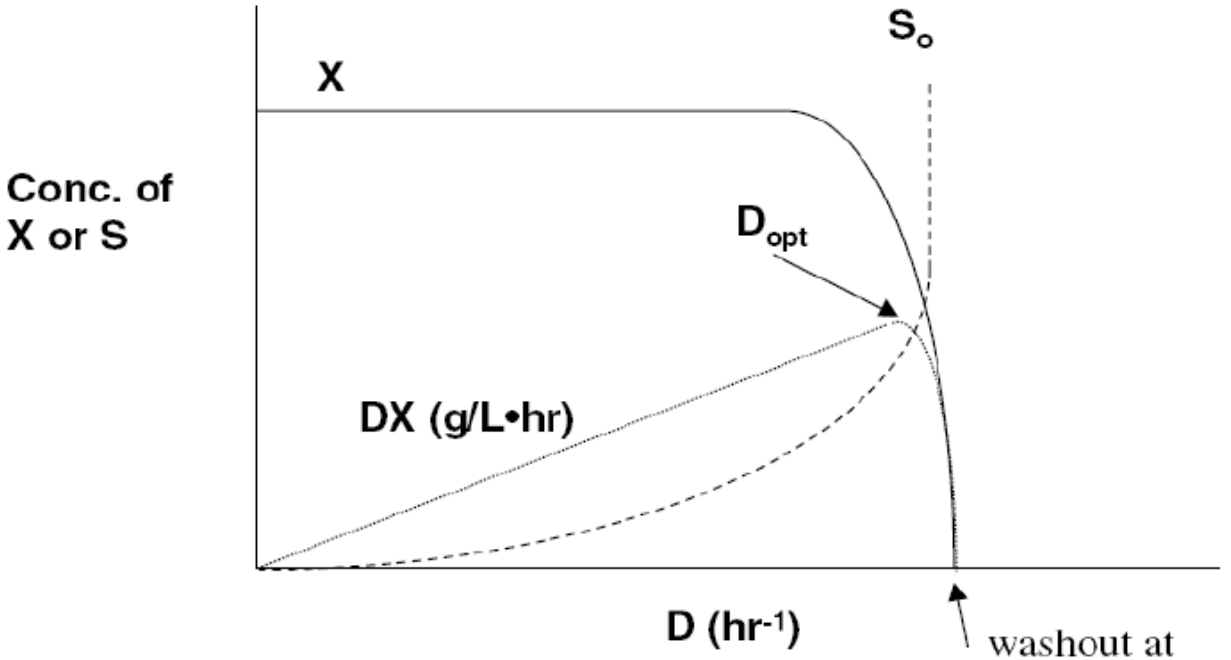
Chemstat response to D



$$D = \frac{\mu_{max} S_o}{K_S + S_o}$$

Bioprocess Development

Fermentation Process

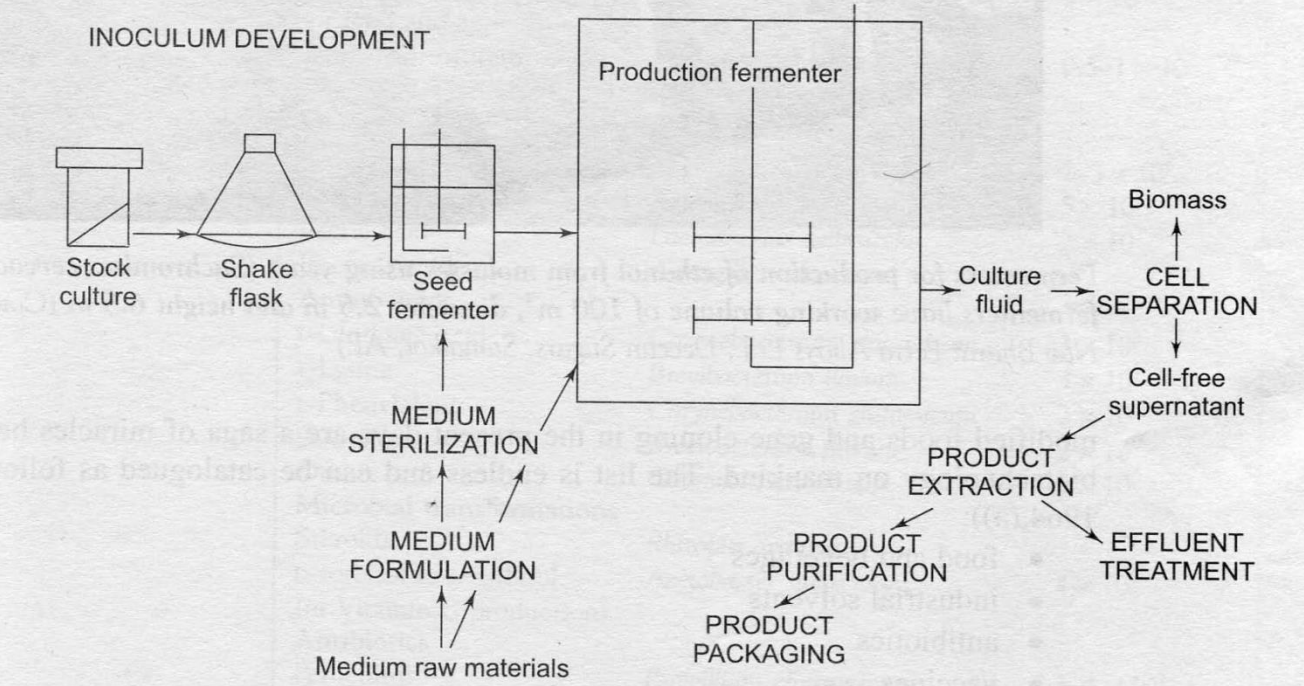


Fig. 5.2

Generalized schematic representation of a typical fermentation process (Reprinted from "Principles of Fermentation Technology", Stanbury, P.F., and Whitaker, A., p.9, Copyright © (1993), with permission from Elsevier.)

Fermentation Products

Major products of biological processing

Fermentation product	*Typical organism used*	*Approximate world market size (kg per year)*
Bulk organics		
Ethanol (non-beverage)	*Saccharomyces cerevisiae*	2×10^{10}
Acetone/butanol	*Clostridium acetobutylicum*	2×10^{6} (butanol)
Biomass		
Starter cultures and yeasts for food and agriculture	Lactic acid bacteria or Bakers' yeast	5×10^{8}
Single-cell protein	*Pseudomonas methylotrophus* or *Candida utilis*	$0.5–1 \times 10^{8}$
Organic acids		
Citric acid	*Aspergillus niger*	$2–3 \times 10^{8}$
Gluconic acid	*Aspergillus niger*	5×10^{7}
Lactic acid	*Lactobacillus delbrueckii*	2×10^{7}
Itaconic acid	*Aspergillus itaconicus*	
Amino acids		
L-Glutamic acid	*Corynebacterium glutamicum*	3×10^{8}
L-Lysine	*Brevibacterium flavum*	3×10^{7}
L-Phenylalanine	*Corynebacterium glutamicum*	2×10^{6}
L-Arginine	*Brevibacterium flavum*	2×10^{6}
Others	*Corynebacterium* spp.	1×10^{6}

Fermentation Products

Microbial transformations		
Steroids	*Rhizopus arrhizus*	
D-sorbitol to L-sorbitol (in Vitamin C production)	*Acetobacter suboxydans*	4×10^7
Antibiotics		
Penicillins	*Penicillium chrysogenum*	$3–4 \times 10^7$
Cephalosporins	*Cephalosporium acremonium*	1×10^7
Tetracyclines (e.g. 7-chlortetracycline)	*Streptomyces aureofaciens*	1×10^7
Macrolide antibiotics (e.g. erythromycin)	*Streptomyces erythreus*	2×10^6
Polypeptide antibiotics (e.g. gramicidin)	*Bacillus brevis*	1×10^6
Aminoglycoside antibiotics (e.g. streptomycin)	*Streptomyces griseus*	
Aromatic antibiotics (e.g. griseofulvin)	*Penicillium griseofulvum*	
Extracellular polysaccharides		
Xanthan gum	*Xanthomonas campestris*	5×10^6
Dextran	*Leuconostoc mesenteroides*	Small

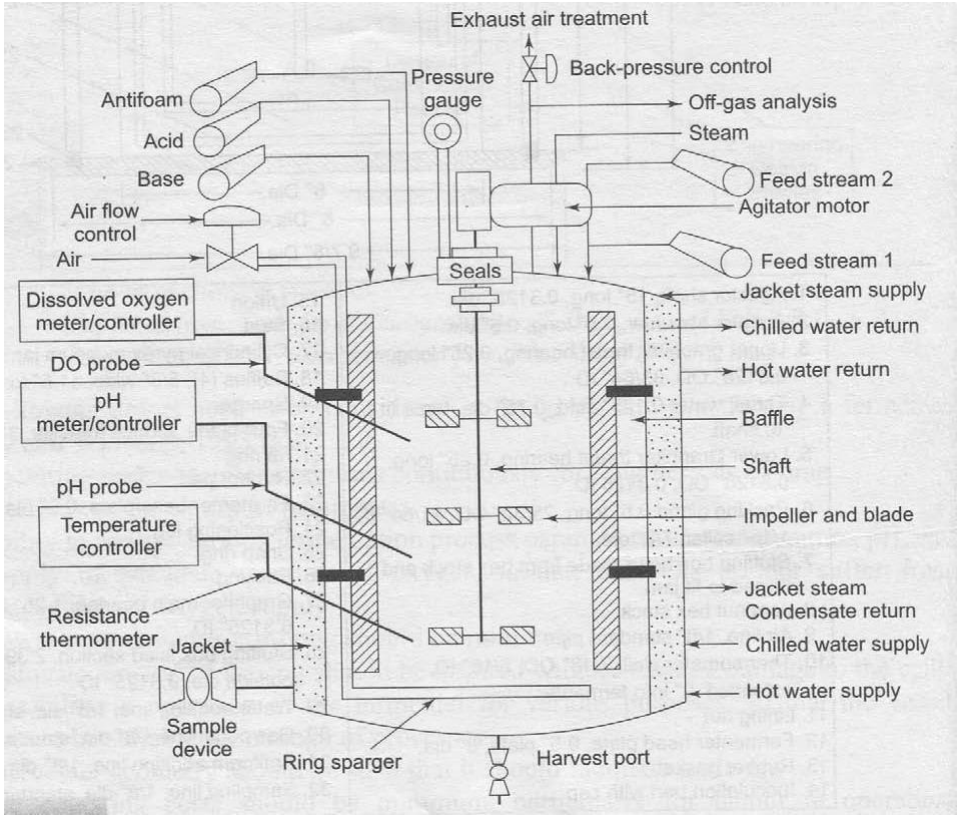
Fermentation Products

Fermentation product	*Typical organism used*	*Approximate world market size (kg per year)*
Nucleotides		
5'-Guanosine monophosphate	*Brevibacterium ammoniagenes*	1×10^5
Enzymes		
Proteases	*Bacillus* spp.	6×10^5
α-Amylase	*Bacillus amyloliquefaciens*	4×10^5
Glucoamylase	*Aspergillus niger*	4×10^5
Glucose isomerase	*Bacillus coagulans*	1×10^4
Pectinase	*Aspergillus niger*	1×10^4
Rennin	*Mucor miehei* or recombinant yeast	1×10^4
All others		5×10^4
Vitamins		
B_{12}	*Propionibacterium shermanii* or *Pseudomonas denitrificans*	1×10^4
Riboflavin	*Eremothecium ashbyii*	
Ergot alkaloids	*Claviceps paspali*	5×10^3
Pigments		
Shikonin	*Lithospermum erythrorhizon* (Plant-cell culture)	60
β-Carotene	*Blakeslea trispora*	

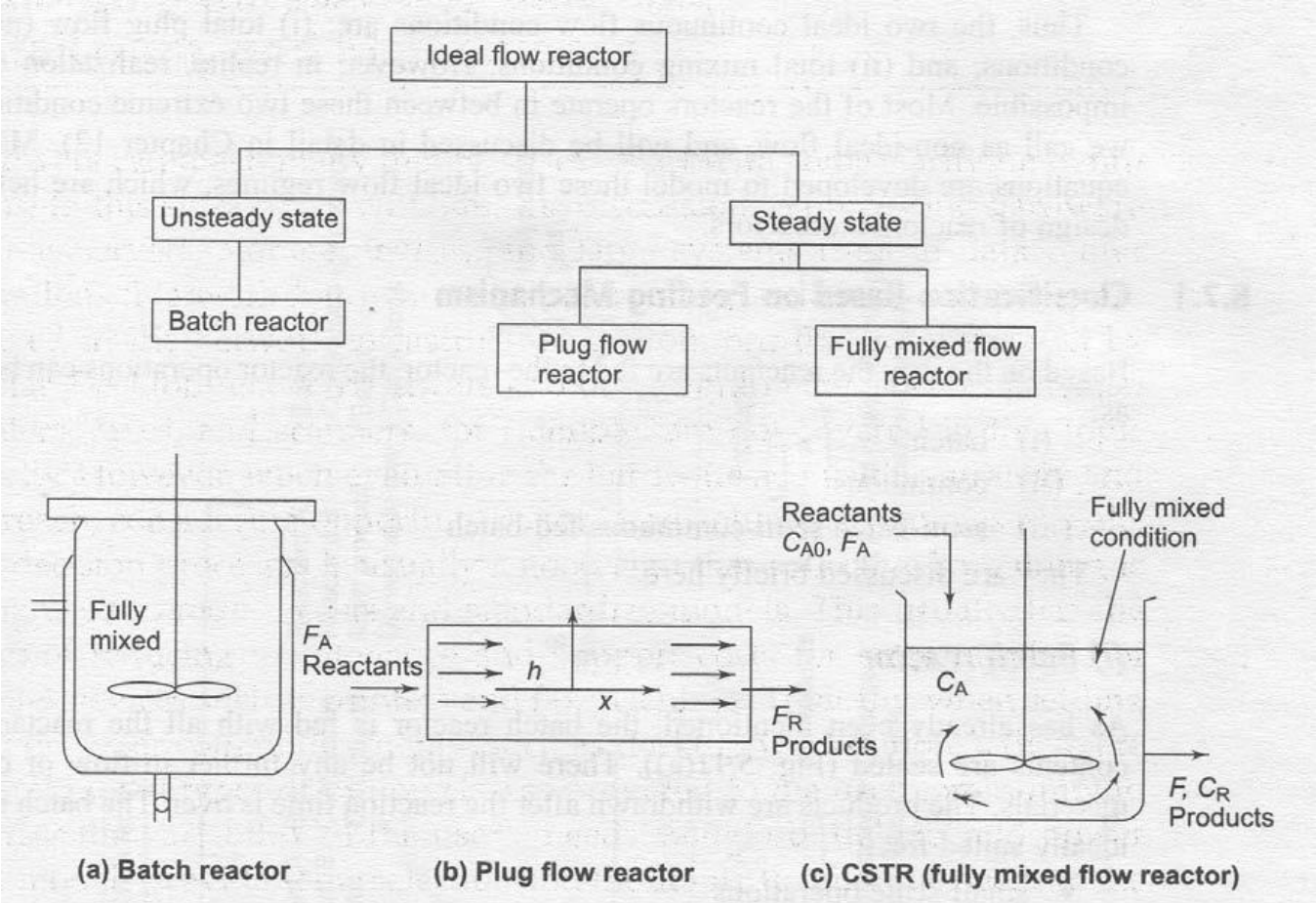
Fermentation Products

Vaccines		
Diphtheria	*Corynebacterium diphtheriae*	< 50
Tetanus	*Clostridium tetani*	
Pertussis (whooping cough)	*Bordetella pertussis*	
Poliomyelitis virus	Live attenuated viruses grown in monkey kidney or human diploid cells	
Rubella	Live attenuated viruses grown in baby-hamster kidney cells	
Hepatitis B	Surface antigen expressed in recombinant yeast	
Therapeutic proteins		<20
Insulin	Recombinant *Escherichia coli*	
Growth hormone	Recombinant *Escherichia coli* or recombinant mammalian cells	
Erythropoietin	Recombinant mammalian cells	
Factor VIII-C	Recombinant mammalian cells	
Tissue plasminogen activator	Recombinant mammalian cells	
Interferon α_2	Recombinant *Escherichia coli*	
Monoclonal antibodies	Hybridoma cells	<20
Insecticides		
Bacterial spores	*Bacillus thuringiensis*	
Fungal spores	*Hirsutella thompsonii*	

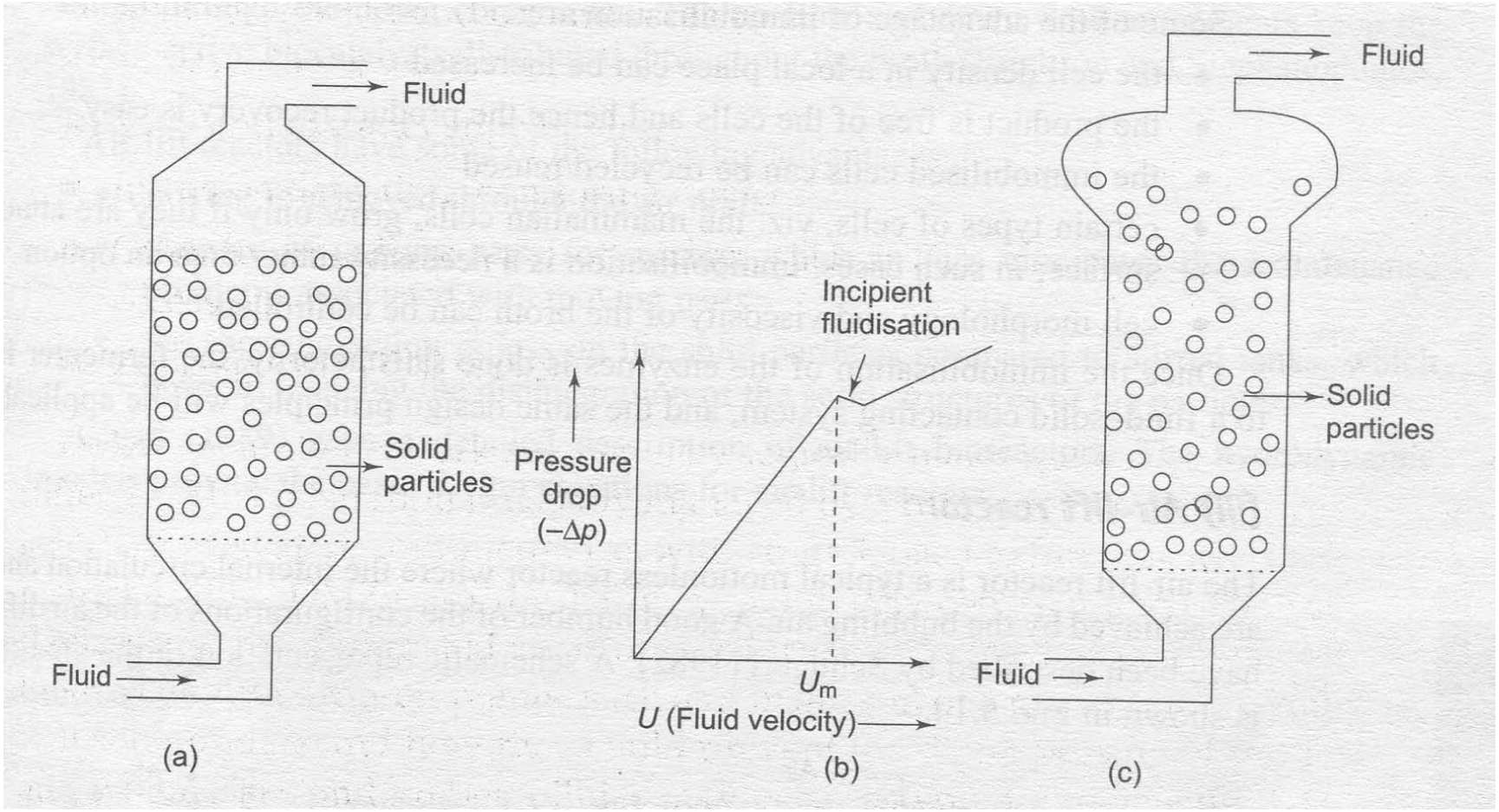
Fermentor


Exhaust air treatment
Back-pressure control
Pressure gauge
Off-gas analysis
Steam
Antifoam
Acid
Base
Feed stream 2
Agitator motor
Air flow control
Air
Feed stream 1
Seals
Jacket steam supply
Dissolved oxygen meter/controller
Chilled water return
DO probe
Hot water return
pH meter/controller
Baffle
Shaft
pH probe
Impeller and blade
Temperature controller
Jacket steam Condensate return
Resistance thermometer
Jacket
Chilled water supply
Hot water supply
Sample device
Ring sparger
Harvest port

Bioreactors



Fluidized Bed Reactor



Air-Lift Reactor