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) $= (1/X)\ (예/dt) = (1/X)\ (\Delta P/\Delta t)$

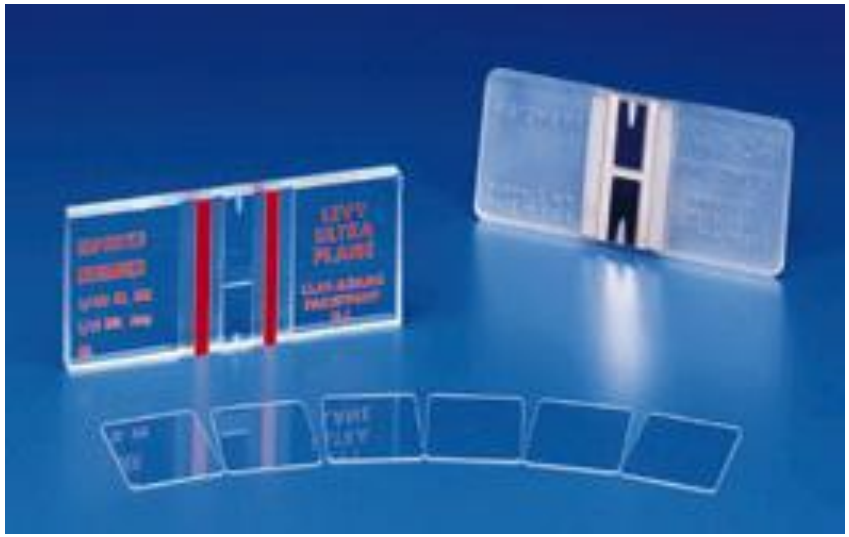
6.1 회분식 생장 (batch growth)

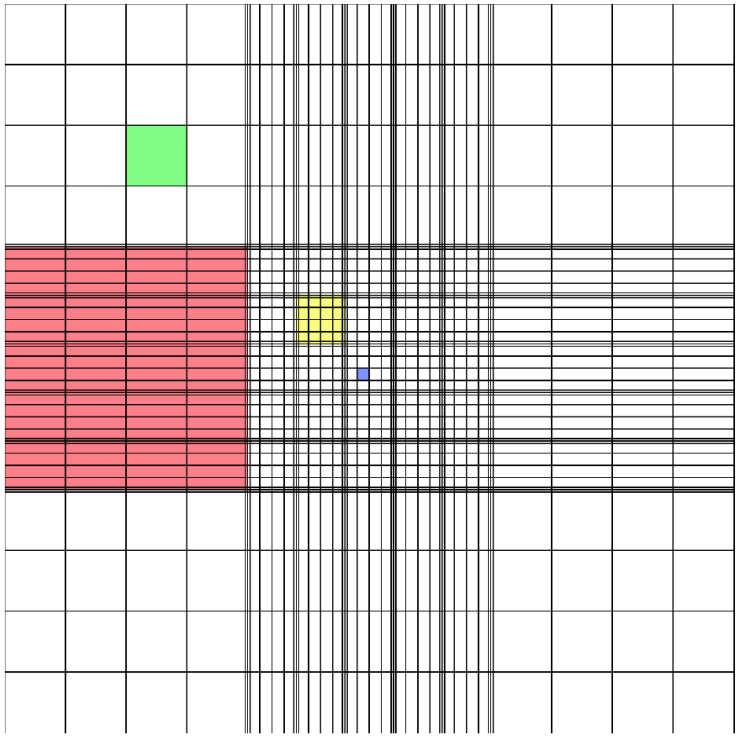
- 초기에 한번 배지를 채운 후 더 이상의 영양물질의 공급이나 제거가 없는 반응기에서 세포 배양
- 단순, 널리 사용
- 세포농도의 정량
  - 세포 수밀도 (number density)
  - 세포 질량농도 (mass concentration)

세포 수밀도의 결정

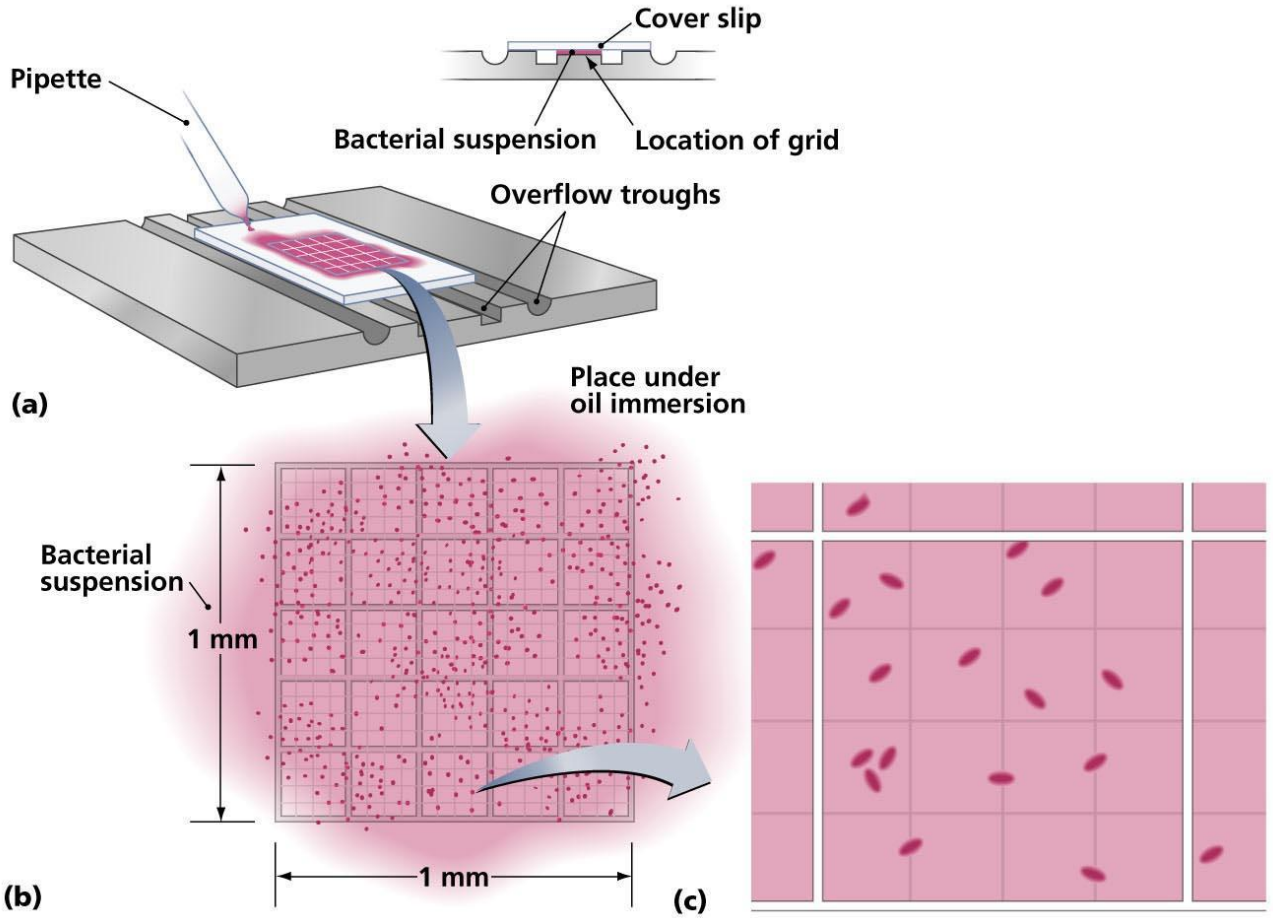
- Petroff-Hausser 슬라이드 (hemocytometer)
  - 간격이 일정하게 정해진 격자구조 (grid)를 culture chamber 위에 놓은 후 현미경을 통해 한칸 당의 세포숫자를 직접 셈
  - 적어도 20칸 이상을 세어 평균
  - 투명배지, 살아 있는 세포와 죽은 세포 구분: 염색
  - 세포가 응집되지 않는 배양계에 적합, 사상곰팡이 X
- 판 계수법 (plate counts)
  - 배지시료 희석, 한천 표면에 퍼뜨린 후 Petri dish 항온배양
    -> 군체형성단위 (CFU)
  - 사상균 X,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

Hemocytometer

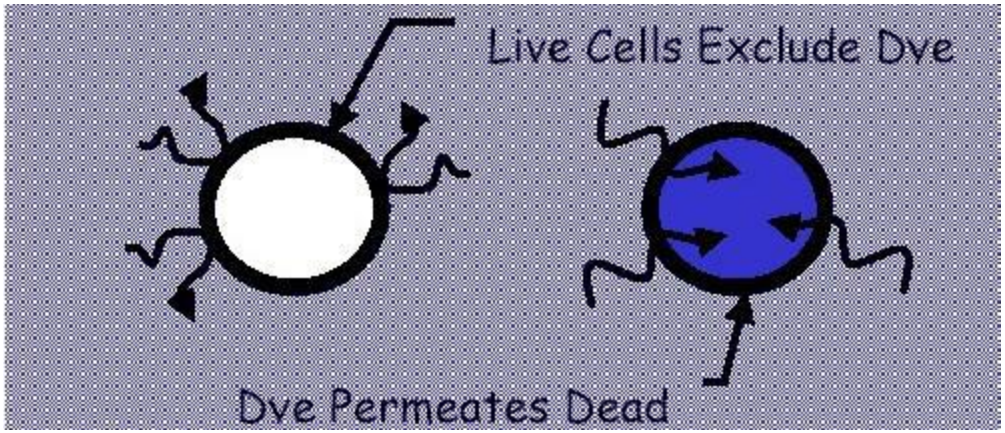




Hemocytometer



Trypan Blue Dye Exclusion Methods



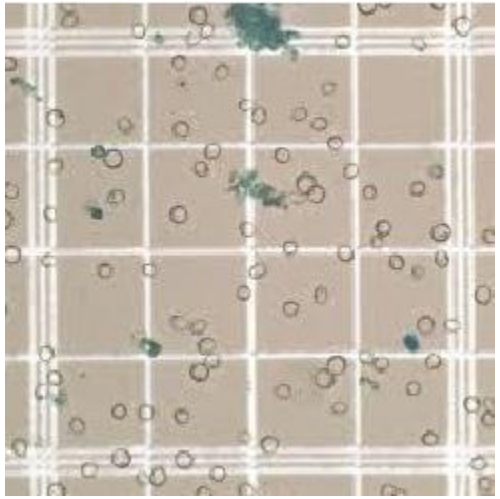
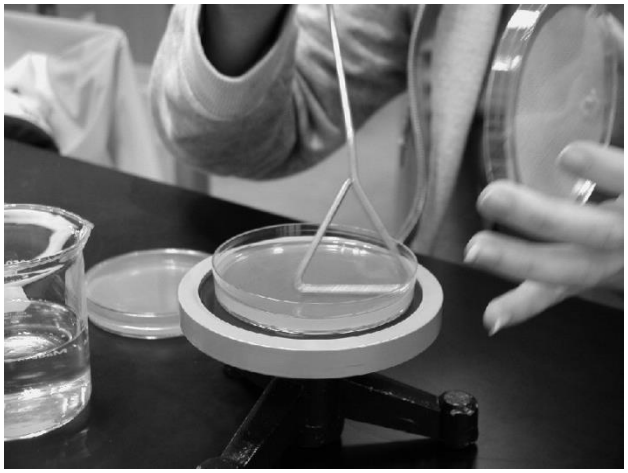
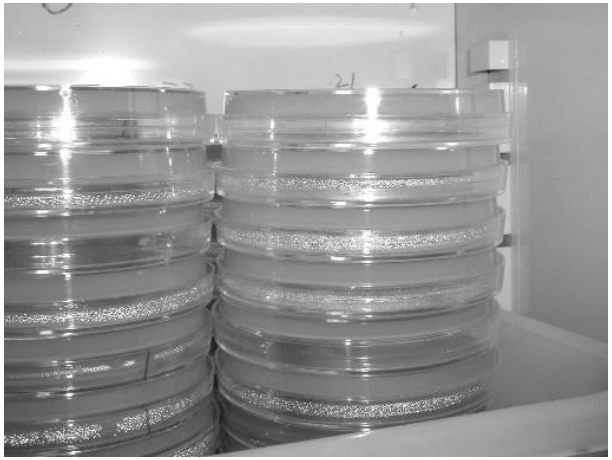


Plate Counts





Particle Counter

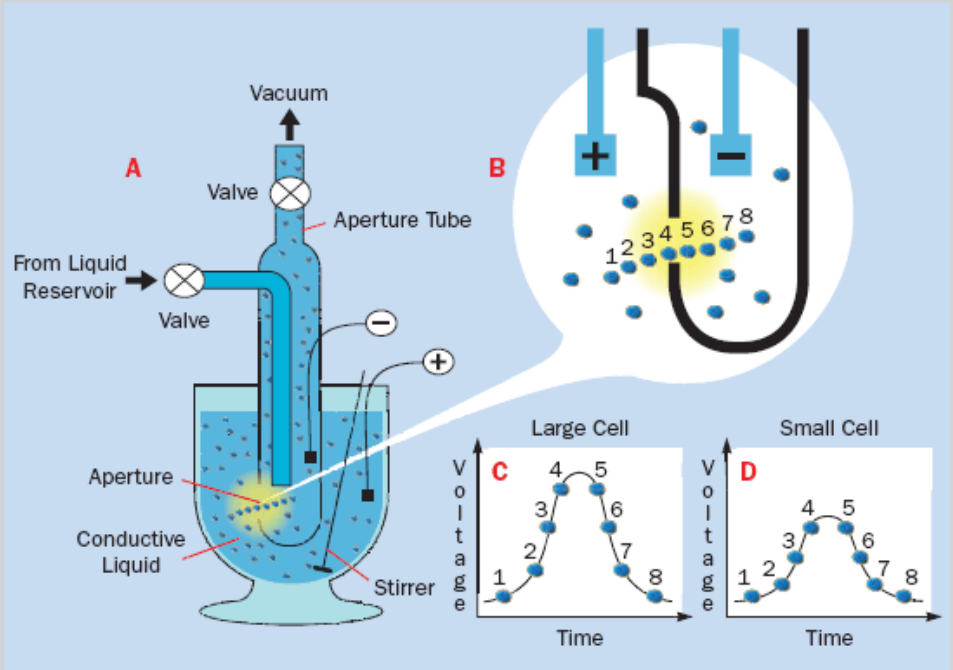


Figure 1. Simplified schematic of a Coulter Counter. As the vacuum pulls the suspension through the aperture in A, electric current flows between the electrodes. As individual particles travel through the sensitive zone (points 1-8) in B, voltage pulses are generated as shown in C and D. The large cell 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

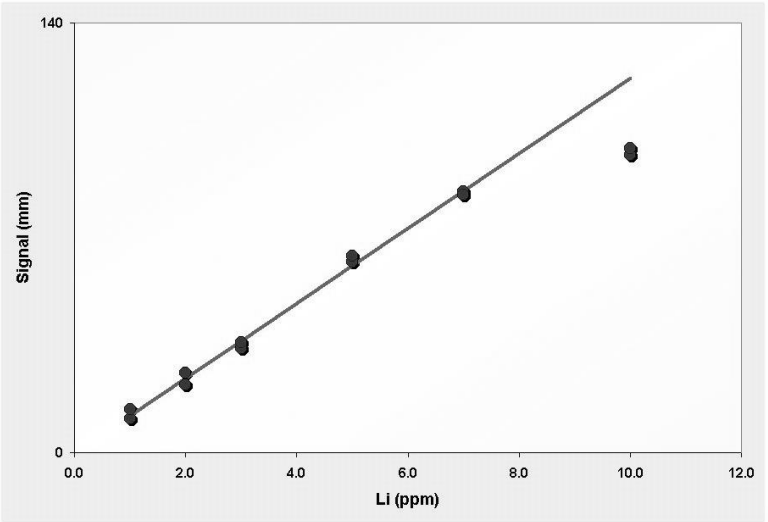
- 세포 건조중량
  - 고형물 (당밀, 셀룰로스, CSL) X
  - 시료 원심분리 or 여과, 완충용액 or 물로 세척, 80 °C, 24 h 건조
- 충전세포 부피 (packed cell volume)
  - 신속히 대충 추산
  - 배양액을 눈금이 매겨진 tapered tube에 넣어 표준 조건에서 원심분리후 세포가 차지하는 부피 측정
- 탁도 (turbidity) or 광학밀도 (optical density)
  - 분광계 (spectrophotometer) 이용
  - 세포가 빛을 흡수하는 정도
  - 배지성분에 의한 흡광을 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
- NADH 농도: 형광 probe
- 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

Yield Coefficients

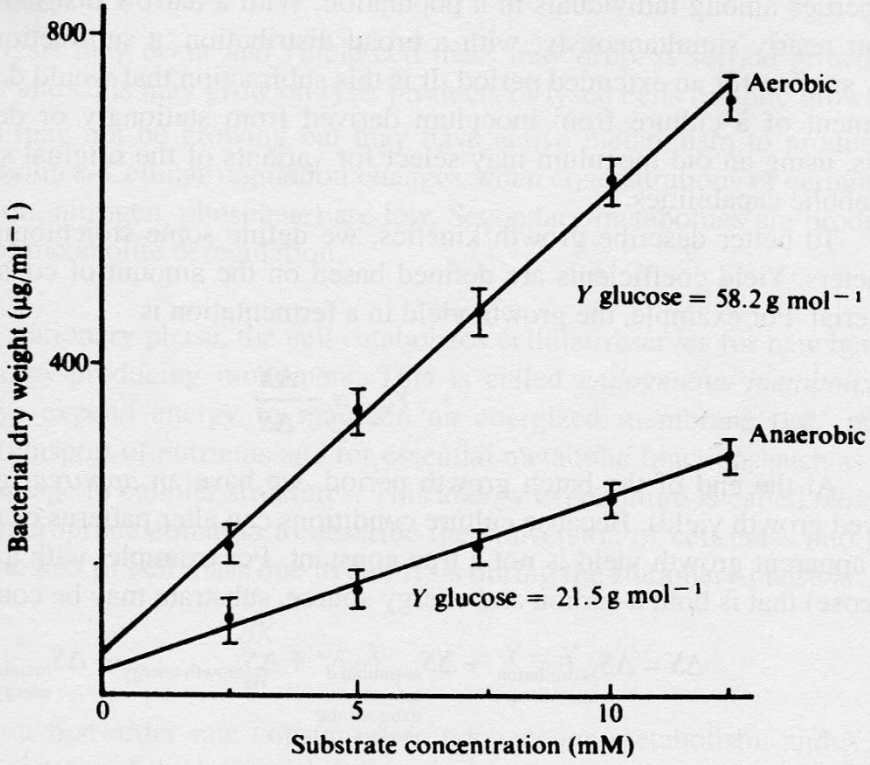


Figure 6.5. Aerobic and anaerobic growth yields of *Streptococcus faecalis* with glucose as substrate. (With permission, from B. Atkinson and F. Mavituna, *Biochemical Engineering and Biotechnology Handbook*, Macmillan, Inc., New York, 1983.)

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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	Glycerol	0.45	41.8	1.16	0.97
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Bioprocess Development

Fermentation Process

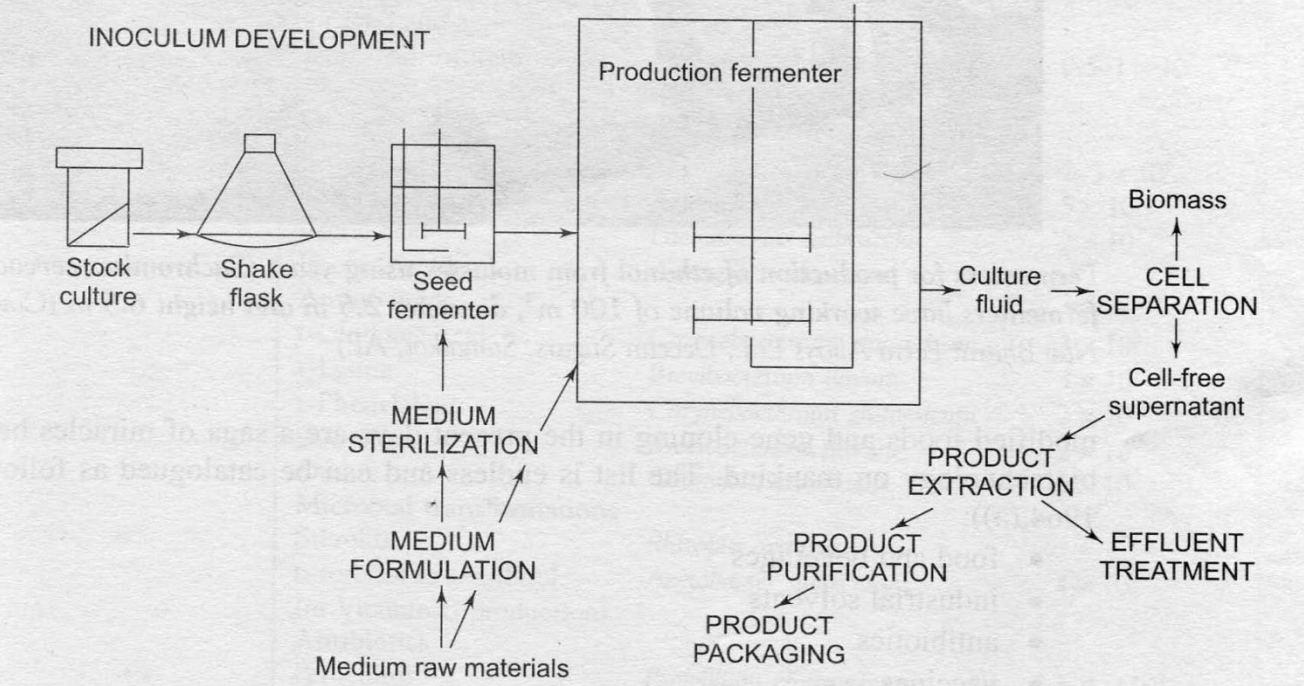


Fig. 5.2

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

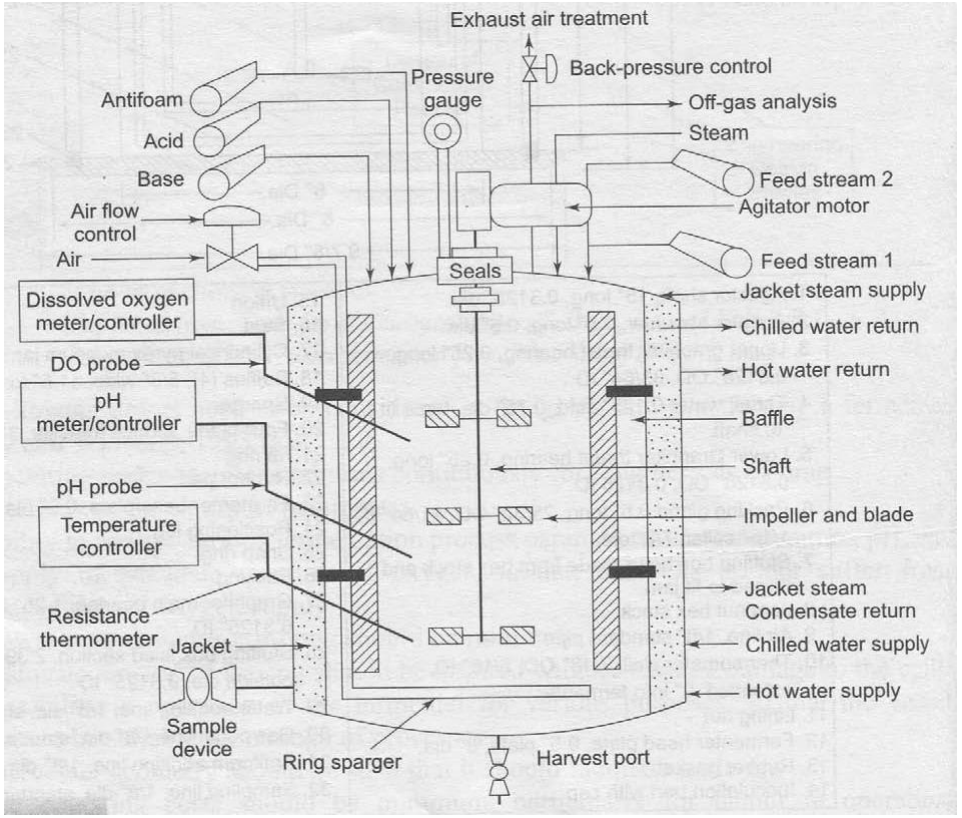
Fermentation Products

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*	

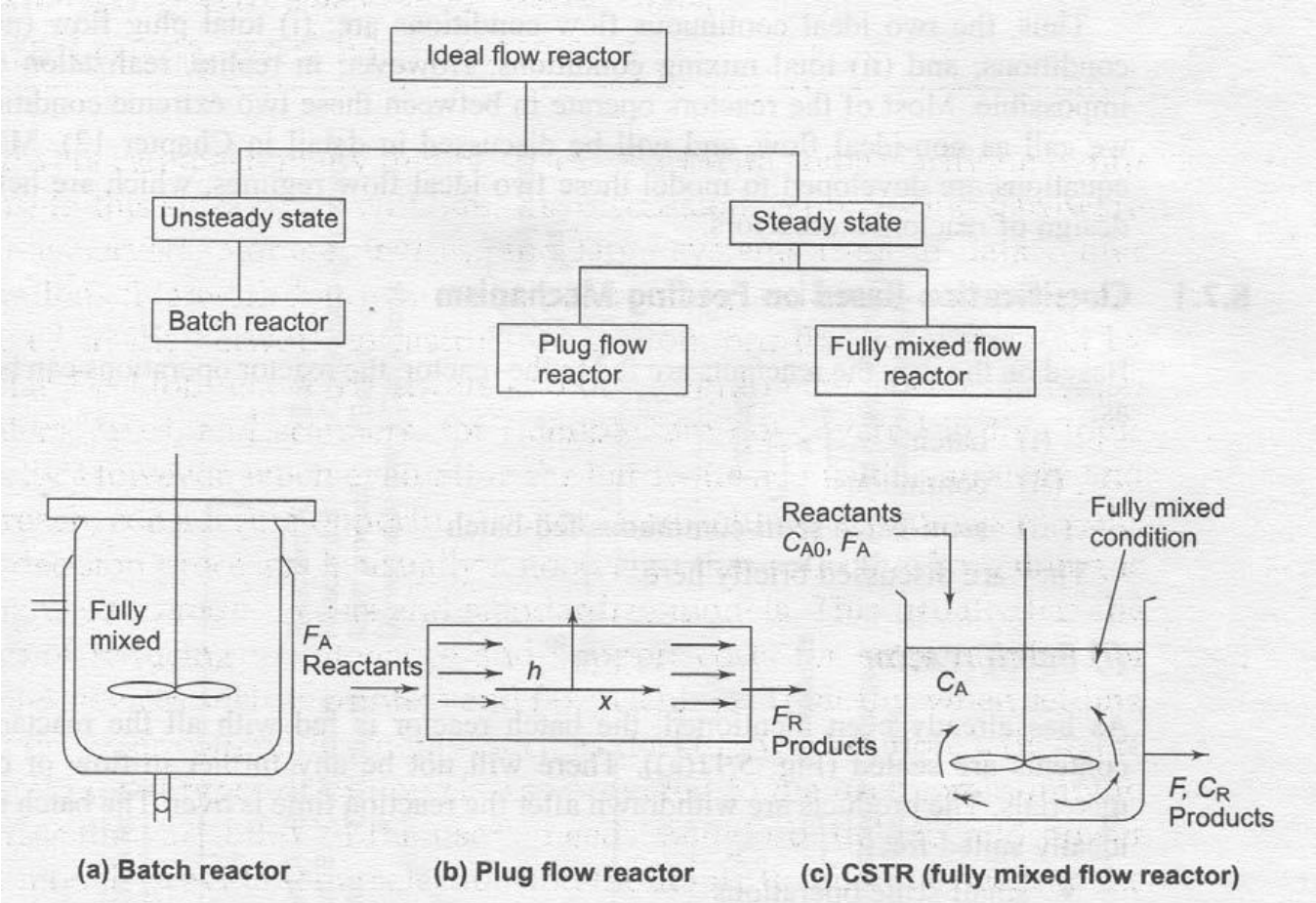
Fermentation Products

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*	

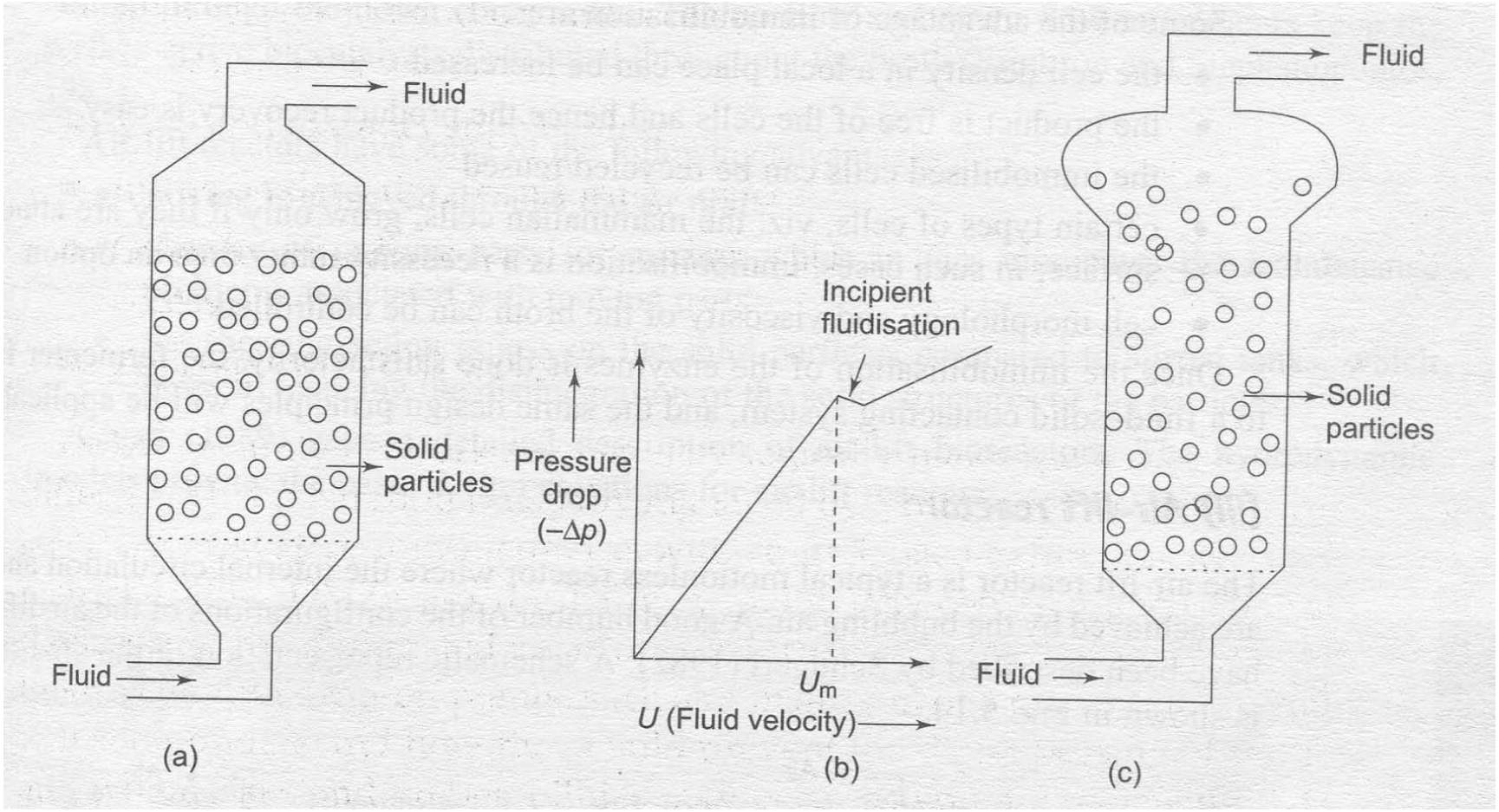
Fermentor


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