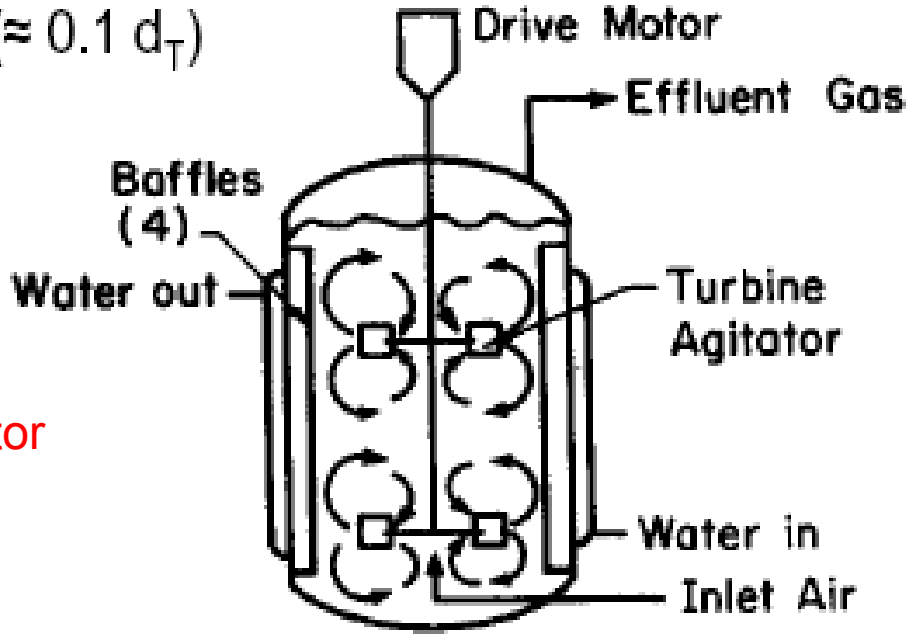
Chapter 10

Bioreactor Type and Sterilization

Type of bioreactors

1. Reactors with Mechanical Agitation
   a) disperse gas bubbles throughout tank
   b) increase residence time of bubbles
   c) shear large bubbles to smaller bubbles
   d) disk type or turbine type ($d_I \approx 0.3\ d_T$)
   e) provide high $k_L a$ values
   f) baffles (4) augment mixing ($\approx 0.1\ d_T$)



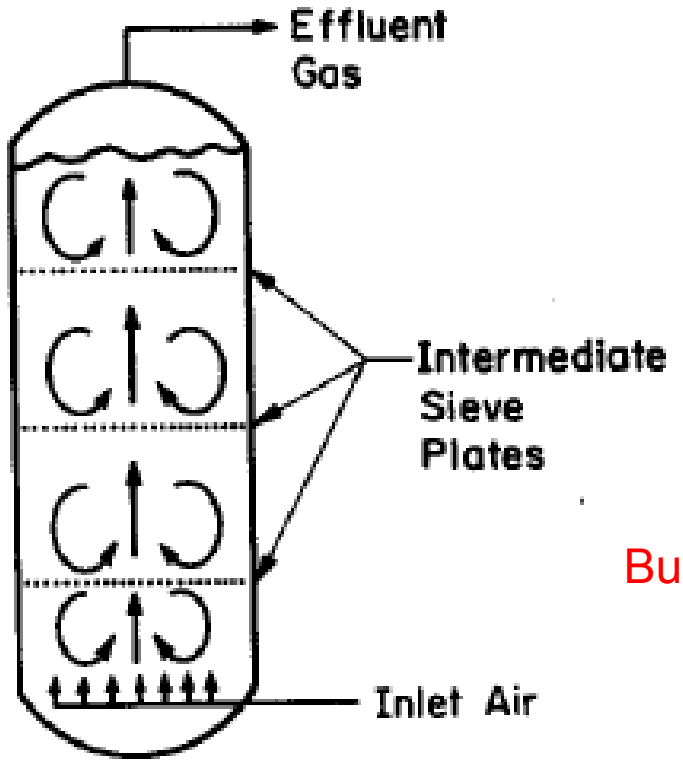
Stirred-tank reactor

Type of bioreactors

2. Bubble Column

a) disperse gas bubbles throughout tank

b) perforated plates enhance gas dispersion and mixing



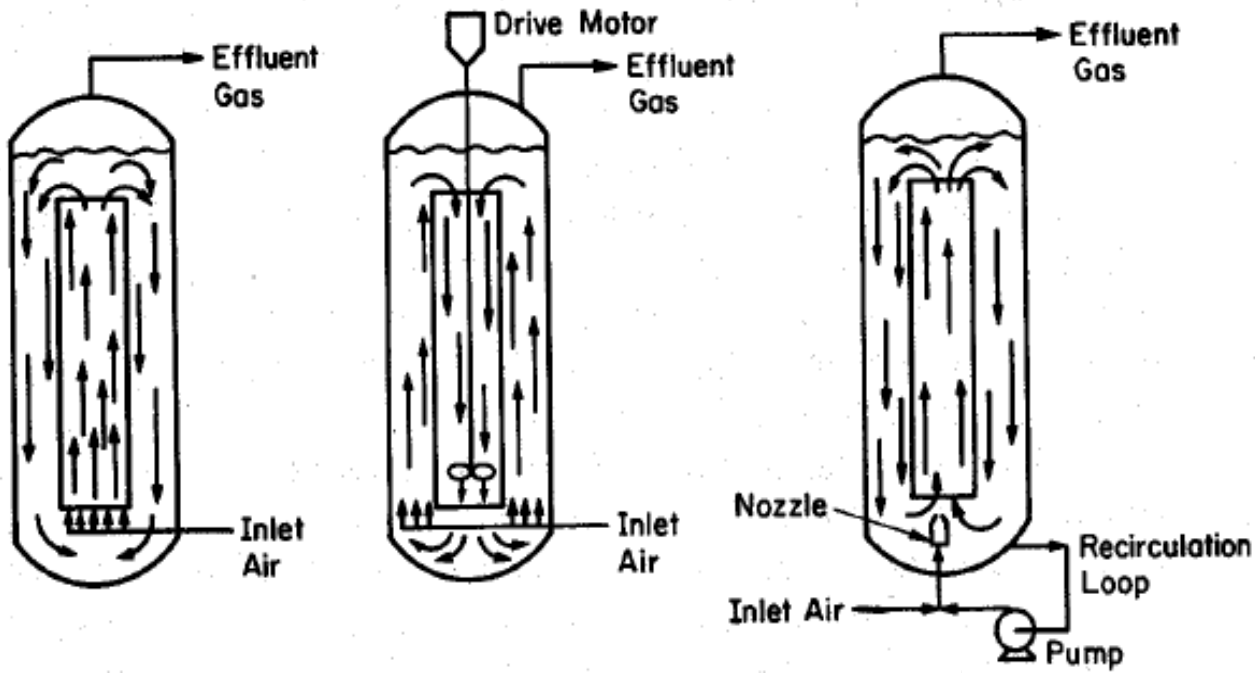
Bubble-column reactor

Type of bioreactors

3. Loop Reactors

a) bubble rising in draft tube causes mixing

b) mixing enhanced by an impeller or a jet pump



Airlift loop reactor

Propeller loop reactor

Jet loop reactor

Materials for bioreactors

Materials of Construction:

Glass Vessels:	Volume < 500 Liters
Stainless Steel Vessels:	All Volumes
	316 ss for vessel
	314 ss for covers & jackets

Aeration

When O_2 transfer is limited or cell density is very high:

$$OUR = OTR$$

$$X\, q_{o2} = k_L\, a\, (C^* - C_L)$$

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)

How to determine $k_L a$

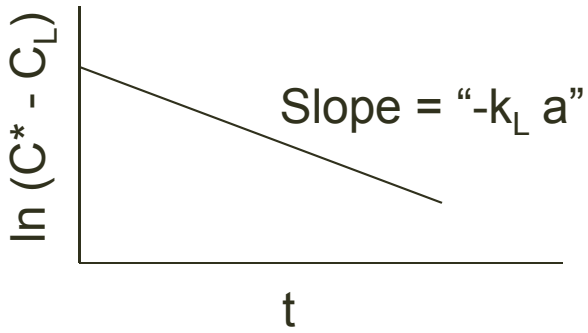
Unsteady state method

1) O_2 is removed from the medium by sparging N2 ($\rightarrow$ DO = 0)
2) Air is introduced into medium and DO is monitored

Unsteady state : $\frac{dC_L}{dt} = OTR = k_L a (C^* - C_L)$

$$-\frac{d(C^* - C_L)}{C^* - C_L} = k_L a \, dt$$

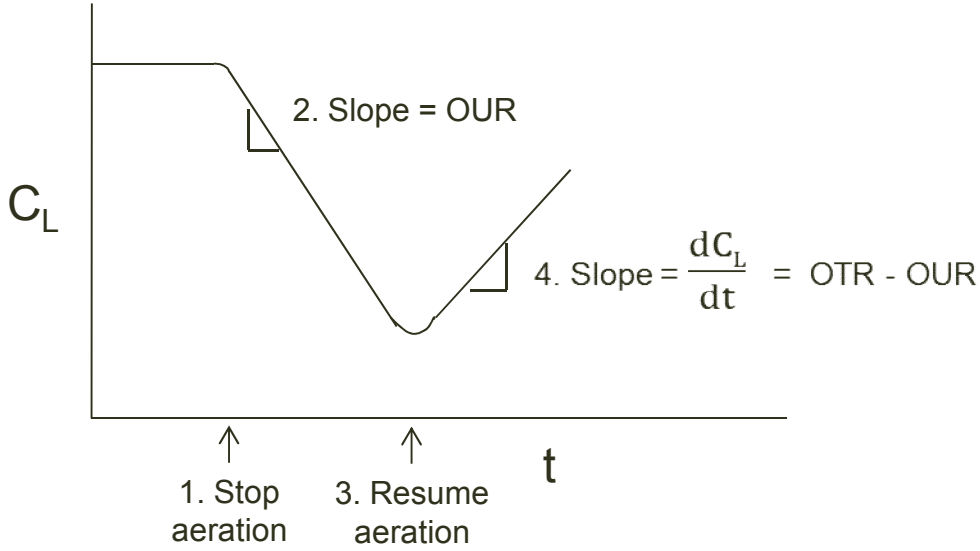
$$\ln (C^* - C_L) = -k_L a \, dt$$



How to determine k_L a

Dynamic method

Bioreactor containing cells



$$\frac{dC_L}{dt} = OTR - OUR = k_L a (C^* - C_L) - X q_{o2}$$

Determined from (4)

5. Finally calculated

known

Measured Using DO probe

= OUR Determined from (2)

Reasons for sterilization

Sterility: *the absence of detectable levels of viable organisms in a culture medium or in a gas*

Reasons for Sterilization

1. Economic penalty is high for loss of sterility
2. Many fermentations must be absolutely devoid of foreign organisms
3. Vaccines must have only killed viruses
4. Recombinant DNA fermentations - exit streams must be sterilized

Sterilization agents

1. Thermal - preferred for economical large-scale sterilizations of liquids and equipment.
2. Chemical - preferred for heat-sensitive equipment
   → ethylene oxide (gas) for equipment
   → 70% ethanol-water (pH=2) for equipment/surfaces
   → 3% sodium hypochlorite for equipment
3. Radiation - uv for surfaces, x-rays for liquids (costly/safety)
4. Filtration
   → membrane filters having uniform micropores
   → depth filters of glass wool

Kinetics of thermal sterilization

Practical considerations:

1. Not all organisms have identical death kinetics.
   → (increasing difficulty; vegetative cells < spores < virus)
2. Individuals within a population of the same organism may respond differently

Kinetics of thermal sterilization

P(t) = Probability that an individual cell is still viable at sterilization time t

N(t) = # of individuals at time t

N_o = # of individuals at t=0

k_d = specific death rate

$$P(t) = \frac{N(t)}{N_o} = \exp(-k_d t)$$

Temperature effects on kinetics of thermal sterilization

Arrhenius Equation

$$k_d = \alpha e^{-E_{od}/RT}$$

α = constant ($time^{-1}$)

R = gas constant

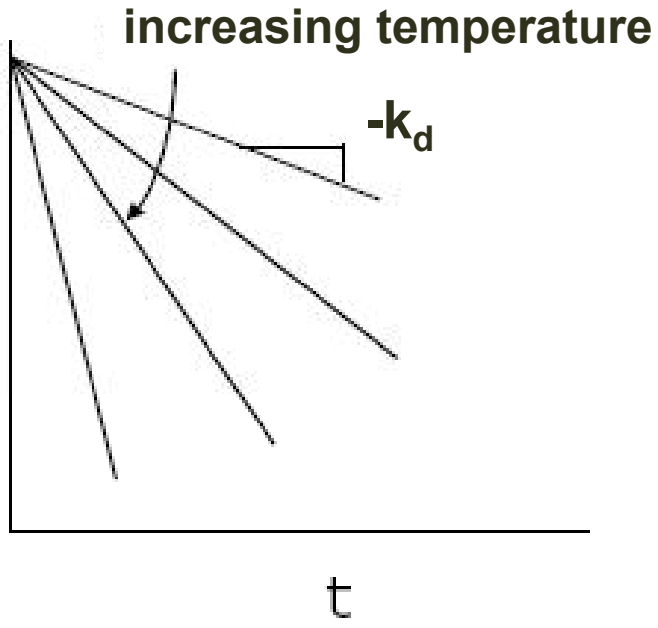
T = absolute temperature

E_{od} = activation energy for death

(50 - 150 kcal / g - mole) for spores

(2 - 20 kcal / g - mole) for vitamins / growth factors

$$\ln \frac{N(t)}{N_o}$$



Population effects on kinetics of thermal sterilization

Most Thermal Sterilizations are at 121°C

Organism	k_d (min^{-1})
Vegetative cells	$>10^{10}$
Spores	0.5 to 5.0

Spores are the primary concern during thermal sterilization

Variables for thermal sterilization

Primary System Variables in Thermal Sterilization

1. Initial concentration of organisms
2. Temperature, T
3. Time (t) of exposure at temperature T.

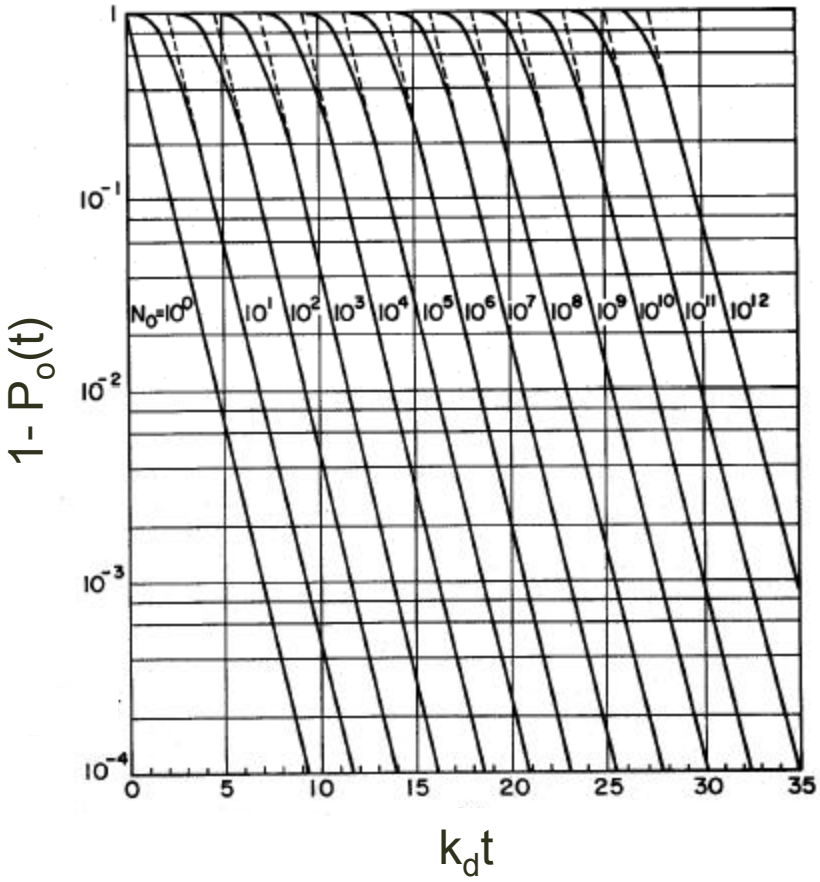
Probability of extinction of total population ;

$P_o(t) = [1-P(t)]^{No}$

Probability of unsuccessful fermentation due to sterilization failure ;

$1- P_o(t) = 1 - [1-P(t)]^{No} = 1 – [1 - \exp(-k_d t)]^{No}$

Sterilization chart



1. Specify 1-$P_o(t)$ which is acceptable (e.g. 10^{-3})

2. Determine N_o in the system.

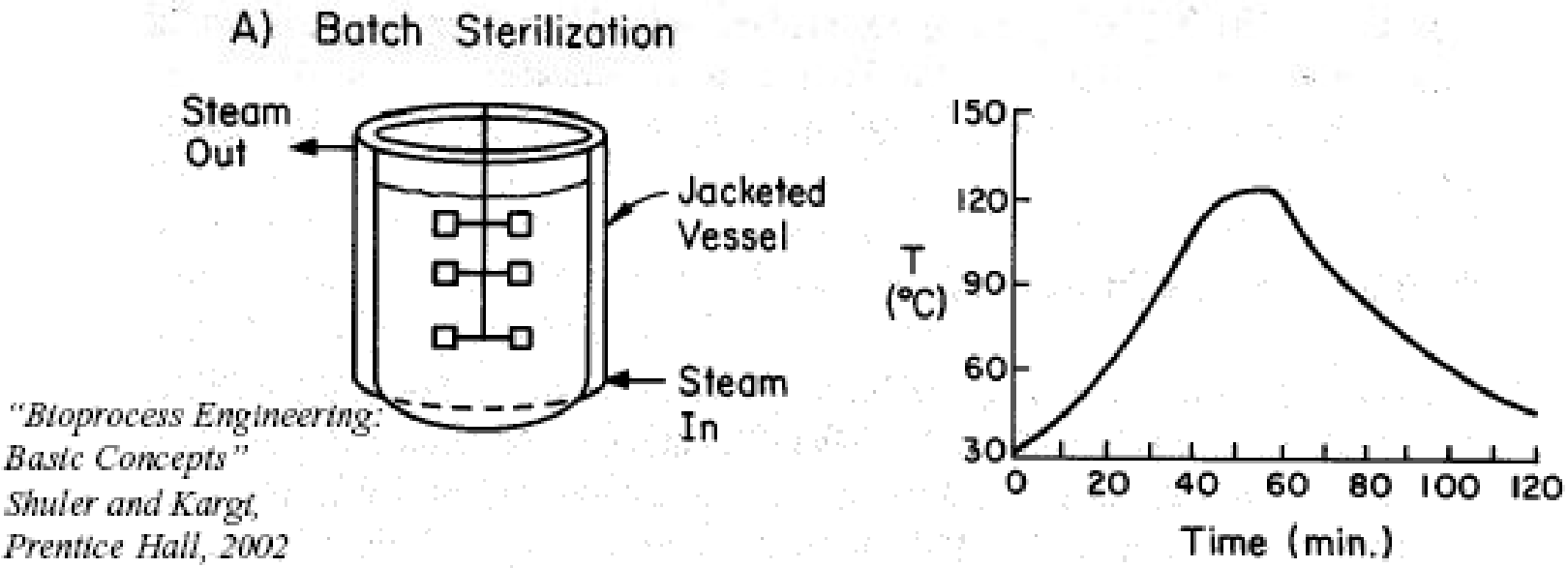
3. Read k_dt from the chart.

4. Knowing k_d for the spores (or cells), obtain the required time, t.

Batch vs. continuous sterilization

Batch

1. Longer heat-up/cool down time
2. Incomplete mixing

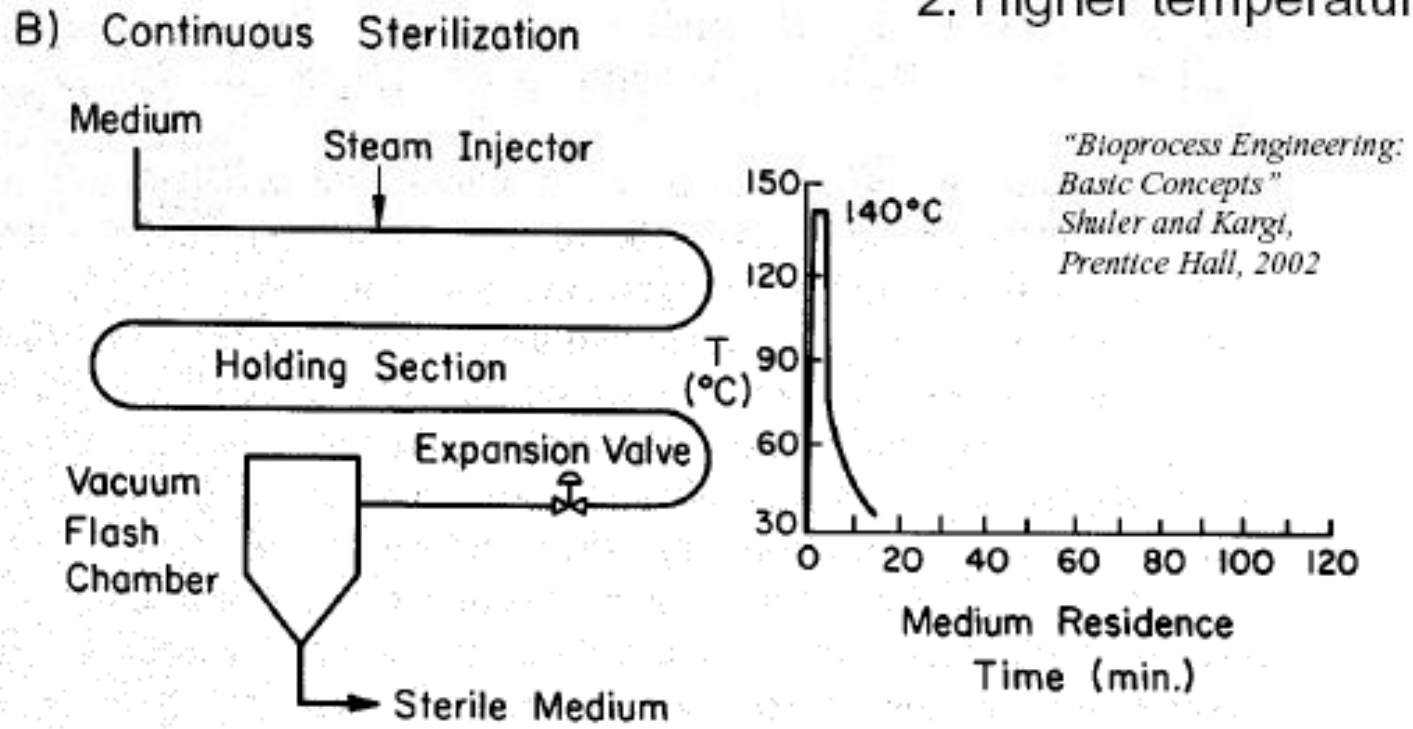


"Bioprocess Engineering: Basic Concepts"
Shuler and Kargi,
Prentice Hall, 2002

Batch vs. continuous sterilization

Continuous

1. Shorter time
2. Higher temperature



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Sterilization of gases

→ aerobic fermentations require 0.1 to 1.0 (L air / (L liquid • min))
→ 50,000 L fermenter requires $7x10^6$ to $7x10^7$ L air/day
→ microorganism concentrations in air are about 1-10 / L air

Methods for Air Sterilization at Inlet

1. Adiabatic compression, 220˚C for 30 seconds
2. Continuous Filtration:
   → depth filters (glass wool filters)
   → surface filters (membrane cartridges)
3. Economics ≈ 25% of production costs for air system

Exit gas must be filtered

→ pathogenic
→ recombinant DNA cells