Chapter 10:
Sterilization and Bioreactor Operation

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Sterilization Methods and Kinetics: 10.4

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 (Death)

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

From Probability Theory:

p(t) = the probability that an individual cell is still viable at time t.

$p(t) = e^{-k_d t}$ (simplest form assuming 1st ord

Kinetics of Thermal Sterilization (cont.)

E[N(t)]= expected value (E) of the numb
of individual organis
at time t after sterilization starts.

$= N_o\, p(t) = N_o\, e^{-k_d t}$

where N_o is the initial number of individuals

$\frac{N(t)}{N_o} = e^{-k_d t}$ or $\ln \frac{N(t)}{N_o} = -k_d t$ survival cu

Temperature Effects on the Kinetics of Thermal Sterilization

Arrhenius Equation

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

α = constant (time^{-1})

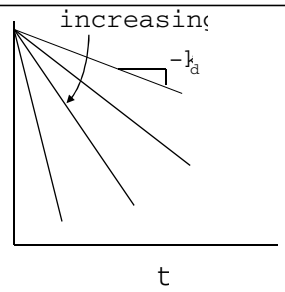
R = gas constant

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



Population Effects on the 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

System Variables for Thermal Sterilization

Primary System Variables in Thermal Sterilization

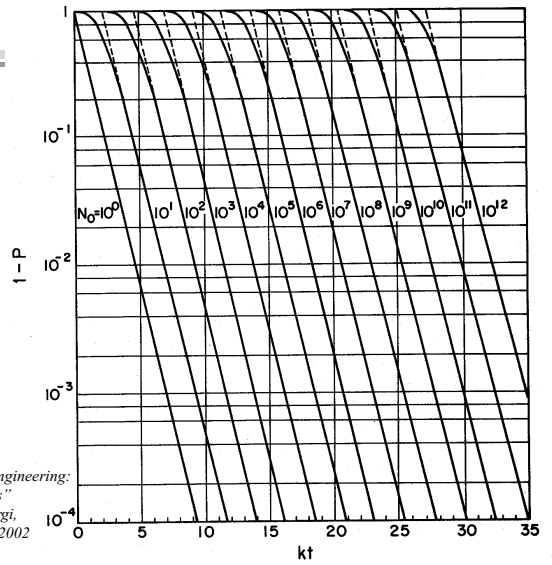
1. Initial concentration of organisms
2. Temperature, T
3. Time (t) of exposure at temperature T.

Probability of an Unsuccessful Ferm[illegible] $P_o(t)$

$[1-P_o(t)] = 1-[1-p(t)]^N$

$= 1-[1-e^{-k_d t}]^{N_o}$ (for a homogeneous popu[illegible]

Sterilization Chart



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System Variables for Thermal Sterilization

Use of Sterilization Charts:

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

2. Determine N_o in the system.

3. Read $k_d t$ from the chart.

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

Scale-up of Sterilization

→ in batch sterilization, scale-up of small-scale sterilization data to a much larger scale will result in unsuccessful sterilization

1-Liter Vessel

n_o = 10^4 spores/L

N_o = (1 L)(n_o)

$[1-P_o(t)] = 1-[1-e^{-k_d t}]^{n_o}$

$= .003$

10^4-L Vessel

n_o = 10^4 spores/L

N_o = (10^4 L)(n_o)

$1-P_o(t) = 1-[1-e^{-k_d t}]^{10^4 n_o}$

$= 1-5\times10^{-14} \approx 1$

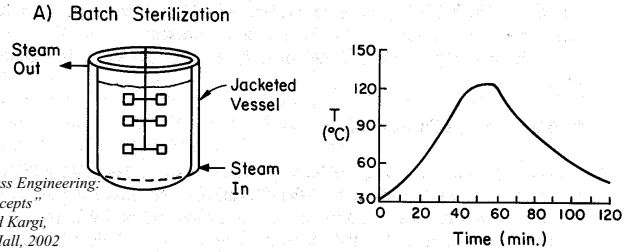
15

15

Batch vs. Continuous Sterilization

Batch

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



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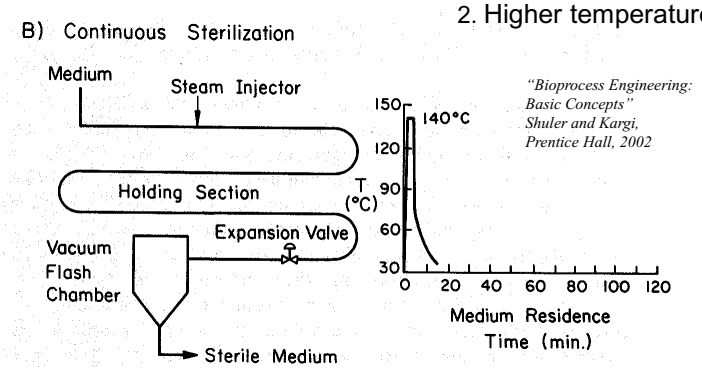
Batch vs. Continuous Sterilization

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

Design and Operation of Bioreactors

Types of Bioreactors

1. Reactors with Mechanical Agitation ***see Fig. 10.1A***
   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 ($d_I \approx 0.3\ d_T$) ***see Fig. 10.3***
   e) provide high k_La values
   f) baffles (4) augment mixing ($\approx 0.1\ d_T$)

2. Bubble Column ***see Fig. 10.1B***
   a) disperse gas bubbles throughout tank
   b) perforated plates enhance gas dispersion and mixing

Figure 10.1A

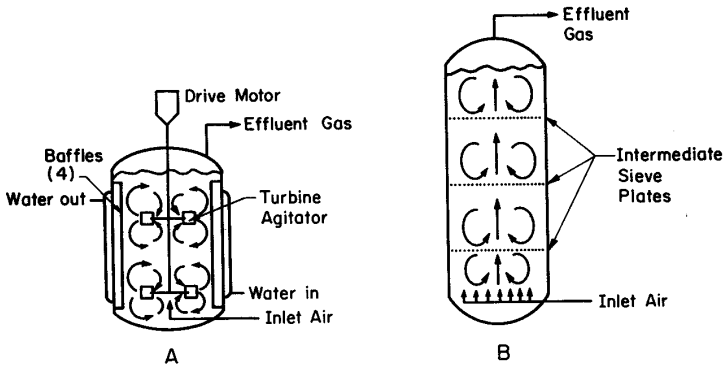

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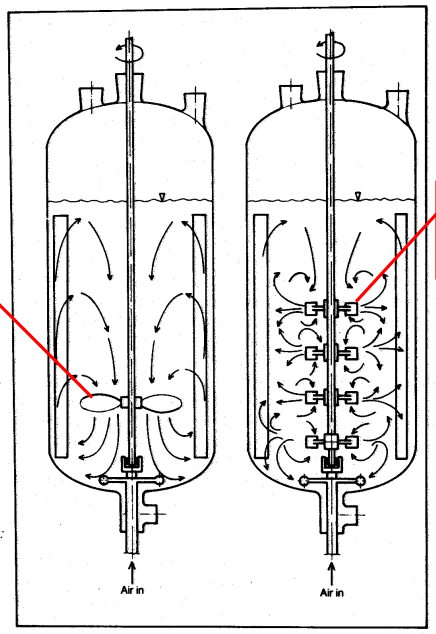
Figure 10.3
(1st Edition)



Axial flow hydrofoil Impeller

- lower energy demand
- comparable gas transfer
- superior axial mixing
- lower shear stress

Rushton Impeller (Disk-type)

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Design and Operation of Bioreactors (cont.)

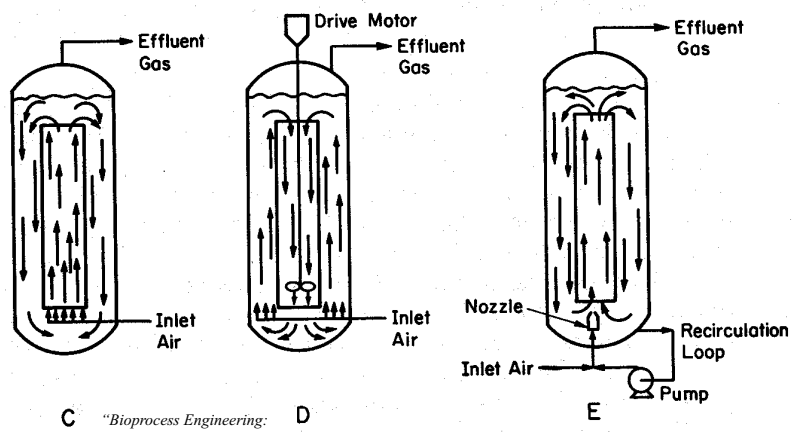
Types of Bioreactors

3. Loop Reactors ***see Fig. 10.1 C, D, E***
   a) bubble rising in draft tube causes mixing
   b) mixing enhanced by an impeller or a jet pump

Materials of Construction:

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

Figure 10.1B



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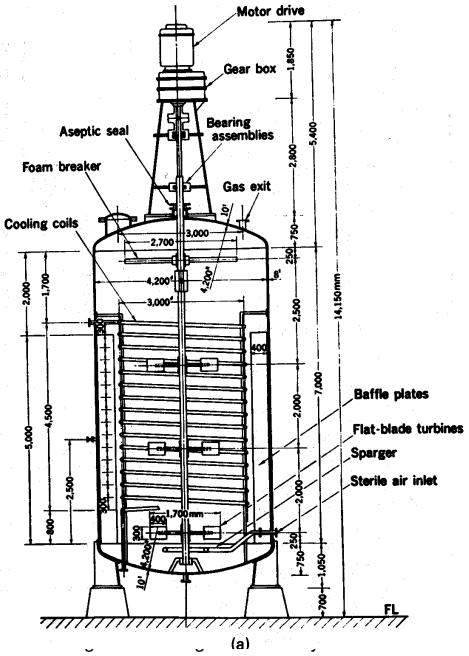
Reactor Geometry and Layout

Figure 10.2:

a) height to diameter ratio of 2 to 3
b) sterile air inlet and sparger
c) baffle plates & impellers
d) cooling coils
e) foam breaker
f) working volume (liquid capacity) ≈ 0.75 vessel volume

Figure 10.2



Height to Diameter Ratio of 2 - 3

$V_L \approx 0.75\ V_R$

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Reactor Types in Industry

Nonstirred/Nonaerated Vessels:

a) most fermentations in terms of total volume

b) food fermentations (beer, wine, diary products)

Stirred / and (or) Aerated Vessels:

a) most fermentations in terms of numbers of units

Aeration and Heat Transfer

Aeration and heat transfer requirements often limit the design of commercial reactors

A multiplication sign

Aeration Design Equation: (OUR = OTR)

Oxygen Uptake Rate: OUR = X □q_{O_2}

X = cell concentration (g cells / L) - ranges from 1 to 5

q_{O_2} = specific O_2 uptake rate (Yield) [mmol O_2 / (g cells □hr)]

(2 to 90; bacteria, yeast, molds)

Table 10.1

TABLE 10.1 Typical Respiration Rates of Microbes and Cells in Culture

Organism	q_{O_2} (mmol O_2/g dw-h)
Bacteria	
E. coli	10–12
Azotobacter sp.	30–90
Streptomyces sp.	2–4
Yeast	
Saccharomyces cerevisiae	8
Molds	
Penicillium sp.	3–4
Aspergillus niger	ca. 3
Plant cells	
Acer pseudoplatanus (sycamore)	0.2
Saccharum (sugar cane)	1–3
Animal cells	
HeLa	$0.4 \frac{\text{mmol } O_2\text{/l-h}}{10^6 \text{ cells/ml}}$
Diploid embryo WI-38	$0.15 \frac{\text{mmol } O_2\text{/l-h}}{10^6 \text{ cells/ml}}$

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Oxygen Transfer Rate

Oxygen Transfer Rate: OTR = $k_L a\,(C^* - C)$

$k_L a$ = volumetric mass transfer coefficient (hr^{-1})

C^* = O_2 concentration in water at the bubble / water interface

$$\cong \frac{P_{O_2}}{H_{O_2}} = \frac{\text{partial pressure of } O_2 \text{ in air (Pa)}}{\text{Henry's Law Constant for } O_2 \text{ (Pa / (mole } O_2 \text{ / L))}}$$

C_L = O_2 concentration in the bulk water (mole O_2 / L)

→ **temperature, pressure, & salt concentration affect C***
→ **vessel geometry, operation, and fluid properties affect $k_L a$**

$k_L a$ for Stirred Tanks

Oxygen Transfer Rate: OTR = $k_L a\,(C^* - C)$

$$k_L a = k\left(\frac{P_g}{V_R}\right)^{0.4} (v_S)^{0.5}\,(N)^{0.5}$$ see equation 10.2a

k = empirical constant (fluid and reactor - specific)
P_g = power requirement for an aerated bioreactor
V_R = bioreactor volume
v_S = superficial gas exit speed = (F_a / A)
F_a = volumetric flow rate of air
A = bioreactor cross - sectional area
N = impeller rotation speed

Units depend upon correlation data

P_g Correlation

$$P_g = K\left(\frac{P_u^2 N D_i^3}{Q_a^{0.56}}\right)^{0.45} \quad \text{or} \quad \frac{P_g}{P_u} = f\left(\frac{Q_a}{N D_i^3}\right)$$

$$N_A = \text{aeration number} = \left(\frac{Q_a}{N D_i^3}\right)$$

K = emperical constant (reactor geometry - specific)

P_u = power requirement for an ungassed bioreactor

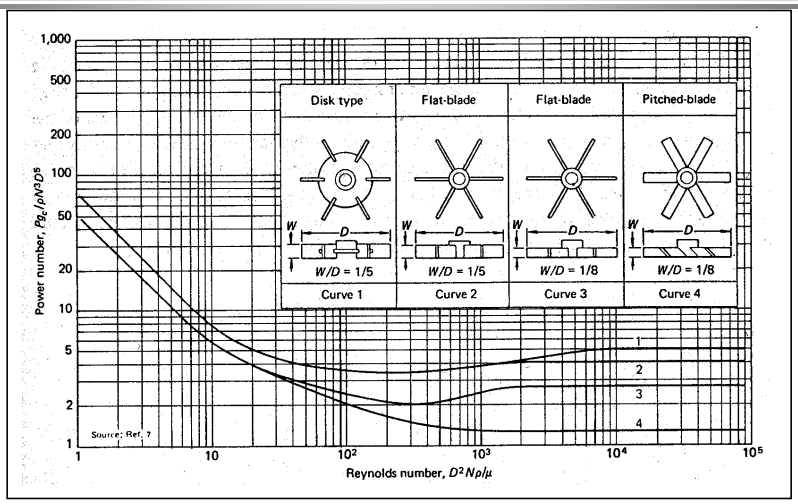
D_i = impeller diameter

Q_a = aeration rate = (F_a / V_R)

Units depend upon correlation data

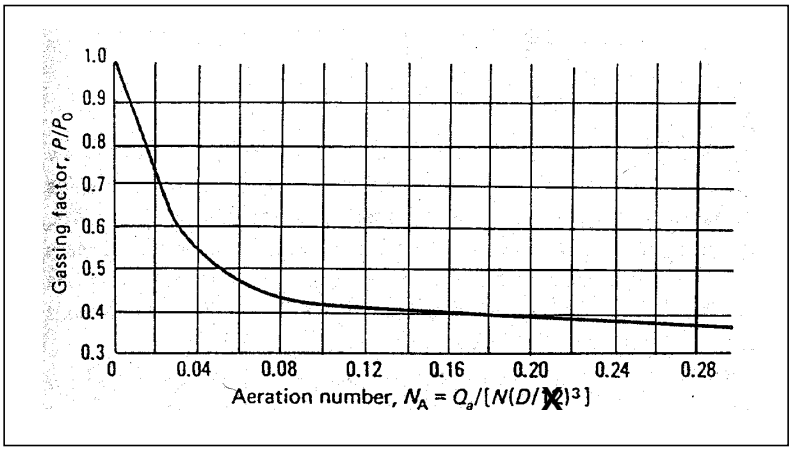
P_u Correlation (Figure 5.20 of Blanch and Clark)

"Biochemical Engineering" Blanch and Clark, Marcel Dekker, 1997



N_A Correlation (Figure 5.22 of Blanch and Clark)

"Biochemical Engineering" Blanch and Clark, Marcel Dekker, 1997



Example Problem

A 10,000 liter (of liquid) bioreactor contains 5 g / L of growing cells

q_{O_2} = 20 mmole O_2 / (g cells □hr) C_L = 1 mg O_2/L

D_T = 2 m, D_i = 1 m, (6 - blade turbine agitator) x 3 blades

For 1 liquid volume per minute aeration rate (air), can the OTR = OUR for N = 100 rpm?

Example Problem Solution

P_u: power requirement for ungassed reactor

$$Re = \text{Reynold's Number} = \frac{\rho_L N D_i^2}{\mu_L}$$

$$\rho_L = 1{,}000 \text{ kg/m}^3 \qquad \mu_L = 10^{-3} \text{ Newton} \cdot \text{s/m}^2$$

$$Re = \frac{\left(1{,}000 \frac{\text{kg}}{\text{m}^3}\right)\left(\frac{100}{60} \text{ s}^{-1}\right)(1^2 \text{ m}^2)\left(1 \frac{\text{Newton}}{(\text{kg} \cdot \text{m/s}^2)}\right)}{10^{-3} \frac{\text{Newton} \cdot \text{s}}{\text{m}^2}}$$

$$= 1.67 \times 10^6$$

Example Problem Solution (cont.)

From Figure 5.20 of Blanch and Clark

$$\text{Power number} = 4 = \frac{P_u}{\rho_L N^3 D_i^5}$$

$$P_u = 4 (\rho_L N^3 D_i^5) \quad \text{for 1 impeller}$$

$$= 4\left[\left(1{,}000 \frac{\text{kg}}{\text{m}^3}\right)\left(\frac{100}{60} \text{ s}^{-1}\right)(1^5 \text{ m}^5)\right] = 1.852 \times 10^4 \frac{\text{kg} \cdot \text{m}^2/\text{s}^2}{\text{s}} \text{ (Watts)}$$

$$P_u \text{ (3 impellers)} = 3\left(1.852 \times 10^4 \frac{\text{kg} \cdot \text{m}^2/\text{s}^2}{\text{s}} \text{ (Watts)}\right) = 5.62 \times 10^4 \text{ Watts}$$

$$= 74.5 \text{ HP}$$

Example Problem Solution (cont.)

P_g: N_A (aeration no.) $= \dfrac{Q_a}{N D_i^3}$

$$N_A = \frac{\left(10{,}000 \dfrac{\text{liters}}{\text{min}}\right)\left(10^{-3} \dfrac{\text{m}^3}{\text{liter}}\right)}{(100\ \text{min}^{-1})(1\ \text{m})^3} = 0.10$$

From Figure 5.22

$$\frac{P_g}{P_u} = 0.42 \quad \Rightarrow \quad P_g = (.42)(5.56\text{x}10^4\ \text{Watts})$$

$$= 2.335\text{x}10^4\ \text{Watts}$$

$$= 31.3\ \text{HP}$$

Example Problem Solution (cont.)

$$k_La\ (\text{mmole } O_2 / (\text{l hr atm}) = 0.60\left[\frac{P_g}{V_R}\left(\frac{\text{HP}}{10^3\ \text{liters}}\right)\right]^{0.4} (v_S)^{0.5}\ (N(\text{rpm}))^{0.5}$$

$$\frac{P_g}{V_R} = \frac{31.3\ \text{HP}}{(10)(10^3\ \text{liters})} = 3.13\ \frac{\text{HP}}{10^3\ \text{liters}}$$

$$v_S = \frac{10^4\ \text{liters/min}\left(10^3 \dfrac{\text{cm}^3}{\text{liter}}\right)}{\dfrac{\pi}{4}(2\ \text{m})^2(10^2\ \dfrac{\text{cm}}{\text{m}})^2} = 318.3\ \frac{\text{cm}}{\text{min}}$$

$$k_La = 0.60(3.13)^{0.4}(318.3)^{0.5}(200)^{0.5} = 169\ (\text{mmole } O_2 / (\text{l hr atm})$$

Example Problem Solution (cont.)

$$OUR = X\,q_{O_2} = \left(5\frac{\text{g cells}}{\text{liter}}\right)\left(20\frac{\text{mmoles } O_2}{\text{g cells}\cdot\text{hr}}\right)$$

$$= 100\ \frac{\text{mmoles } O_2}{\text{liter}\cdot\text{hr}}$$

$$OTR = k_L a(P_{O_2} - P^*)$$

$$P^* \text{ for } C_L = 1\ \frac{\text{mg } O_2}{\text{liter}} = H_{O_2} C_L$$

$$= \left(\frac{0.21\text{ atm}}{8\ \frac{\text{mg } O_2}{\text{liter}}}\right)\left(1\frac{\text{mg } O_2}{\text{liter}}\right) = 0.0263\text{ atm}$$

Example Problem Solution (cont.)

$$OTR = k_L a(P_{O_2} - P^*)$$

$$= 169\ \frac{\text{mmoles } O_2}{\text{liter}\cdot\text{hr}\cdot\text{atm}}\ (0.21 - 0.0263)\text{ atm}$$

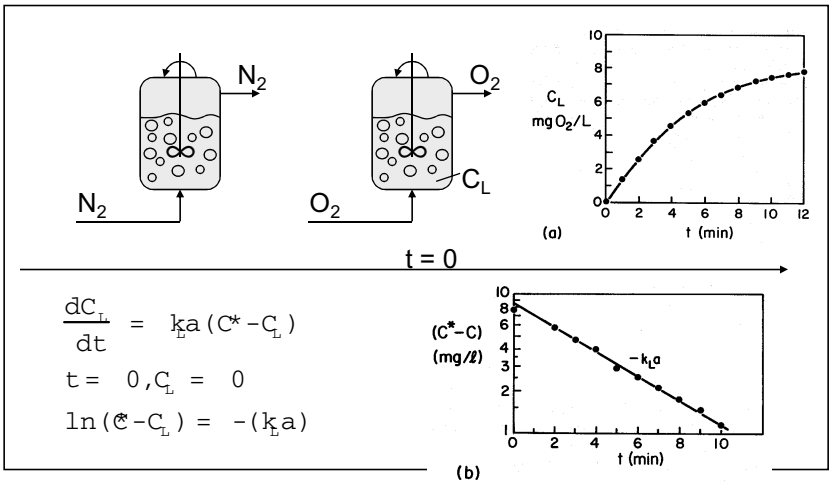
$$= 31.05\ \frac{\text{mmoles } O_2}{\text{liter}\cdot\text{hr}}$$

Since OUR > OTR, we must modify the bioreactor operation in order to bring them into balance

- ***increase N***
- ***use pure O_2 rather than air.***

Measurement of OTR

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



$$\frac{dC_L}{dt} = k_La(C^*-C_L)$$

$$t = 0, C_L = 0$$

$$\ln(C^*-C_L) = -(k_La)$$

Heat Generation Rate: Aerobic Growth

$$Q_{GR} \approx 0.12 \text{ (OUR)}$$

$\left(\frac{kca}{L \cdot hr}\right)$ $\left(\frac{mmol\ O_2}{L \cdot hr}\right)$

Heat Generation Rate: Agitation

$$Q_{agit} = \frac{P_g \text{ (power input aerated}}{V_R \text{ (working volume of r}}$$

$$\approx \left(\frac{1 \text{ hp}}{100 \text{ ga}}\right)$$

Heat Balance

$\text{HRR (Heat Removal Rate)} = U\,A\,\Delta T_{LM}$

$U = \text{overall heat transfer coefficient}$

$A = \text{surface area of heat transfer surface}$

$\Delta T_{LM} = \text{log mean temperature difference between the bioreactor fluid and cooling fluid}$

$$= \frac{(T - t_1) - (T - t_2)}{\ln[(T - t_1)/(T - t_2)]}$$

$T = \text{bioreactor fluid temperature}$

$t_1 = \text{cooling water inlet temperature}$

$t_2 = \text{cooling water outlet temperature}$