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 economic 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:**

\[ p(t) = e^{-kt} \] (simplest form assuming 1st order kinetics)
Kinetics of Thermal Sterilization (cont.)

\[ E(N(t)) = \text{expected value (E) of the number of individual organisms (N(t)) present at time } t \text{ after sterilization starts.} \]

\[ = N_0 \cdot p(t) = N_0 \cdot e^{-k_d t} \]

where \( N_0 \) is the initial number of individuals

\[ \frac{N(t)}{N_0} = e^{k_d t} \quad \text{or} \quad \ln \frac{N(t)}{N_0} = -k_d t \]

"survival curve"

Temperature Effects on the Kinetics of Thermal Sterilization

**Arrhenius Equation**

\[ k_d = \alpha e^{-E_{ad}/RT} \]

\( \alpha = \text{constant (time}^{-1}) \)

\( R = \text{gas constant} \)

\( T = \text{absolute temperature} \)

\( E_{ad} = \text{activation energy for death} \)

(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

<table>
<thead>
<tr>
<th>Organism</th>
<th>$k_d$ (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative cells</td>
<td>&gt;$10^{10}$</td>
</tr>
<tr>
<td>Spores</td>
<td>0.5 to 5.0</td>
</tr>
</tbody>
</table>

*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 Fermentation ($1-P_o(t)$)

\[
(1-P_o(t)) = 1 - (1 - p(t))^N_0 \\
= 1 - (1 - e^{-k_d t})^N_0 \quad \text{(for a homogeneous population)}
\]
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

\[
\text{1-Liter Vessel} \\
\begin{align*}
    n_0 &= 10^4 \text{ spores/L} \\
    N_0 &= (1 \text{ L})(n_0) \\
    (1-P_0(t)) &= 1 - (1 - e^{-kdt})n_0 \\
    &= .003
\end{align*}
\]

\[
\text{100-Liter Vessel} \\
\begin{align*}
    n_0 &= 10^4 \text{ spores/L} \\
    N_0 &= (10^2 \text{ L})(n_0) \\
    (1-P_0(t)) &= 1 - (1 - e^{-kdt})10^2n_0 \\
    &= 1 - 5 \times 10^{-14} \approx 1
\end{align*}
\]

Batch vs. Continuous Sterilization

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

**Continuous**
1. Shorter time
2. Higher temperature
Batch vs. Continuous Sterilization

**Continuous Sterilization**

- **B) Continuous Sterilization**
- Medium
- Steam Injector
- Holding Section
- Vacuum Flash Chamber
- Expansion Valve
- Sterile Medium

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

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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 7x10⁶ to 7x10⁷ 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

David R. Shonnard Michigan Technological University
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 ($d_t \approx 0.3 d_i$)  
   see Fig. 10.3
   e) provide high $k_{La}$ values
   f) baffles (4) augment mixing ($= 0.1 d_t$)

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

Figure 10.1A
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  

David R. Shonnard  Michigan Technological University
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

\[ V_L \approx 0.75 \, V_R \]


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

Aeration Design Equation: \( \text{OUR} = \text{OTR} \)

Oxygen Uptake Rate: \( \text{OUR} = X q_{O_2} \)

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

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

(2 to 90; bacteria, yeast, molds)

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Table 10.1

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

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

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### Oxygen Uptake 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
  
  \[ C^* = \frac{P_{O_2}}{H_{O_2}} \]
  
  *Henry’s Law Constant for \( O_2 \) (Pa / (mole \( O_2 \) / 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 = \( k_L a \ (C^* - C) \)

\[
k_L a = k \left( \frac{P_g}{V_R} \right)^{0.4} (v_s)^{0.5} (N)^{0.5} \text{ see equation 10.2a}
\]

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

**Units depend upon correlation data**
**P_g Correlation**

\[ P_g = K \left( \frac{P_u}{Q_a^{0.56}} \right)^{0.45} \]  

or \[ \frac{P_g}{P_u} = f \left( \frac{Q_a}{N D_i^3} \right) \]

\( N_A \) = aeration number  
\( K \) = empirical 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)

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

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

\( q_{O_2} = 20 \text{ mmole O}_2 / (\text{g cells \cdot hr}) \)

\( D_T = 2 \text{ m}, \quad D_i = 1 \text{ m}, \) (6 - blade turbine agitator) x 3 blades

*For 1 liquid volume per minute aeration rate (air), can the OTR = OUR for \( N = 100 \text{ rpm} \)?*
Example Problem Solution

\[ P_u^* \text{: power requirement for ungassed reactor} \]

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

\[ \rho_L = 1,000 \text{ kg/m}^3 \quad \mu_L = 10^3 \text{ Newton s/m}^2 \]

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

\[ = 1.67 \times 10^6 \]

Example Problem Solution (cont.)

From Figure 5.20 of Blanch and Clark

Power number = 4 = \( \frac{P_u}{\rho_L N^0 D_i^0} \)

\[ P_u = 4 (\rho_L N^0 D_i^0) \text{ for 1 impeller} \]

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

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

\[ = 74.5 \text{ HP} \]
Example Problem Solution (cont.)

\[ P_g: N_A \text{ (aeration no.)} = \frac{Q_g}{N D} \]

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

From Figure 5.22

\[ \frac{P_g}{P_u} = 0.42 \quad \Rightarrow \quad P_g = (0.42)(5.56 \times 10^4 \text{ Watts}) = 2.335 \times 10^4 \text{ Watts} = 31.3 \text{ HP} \]

Example Problem Solution (cont.)

\[ k_{La} \text{ (mmole O}_2/(l \cdot \text{hr} \cdot \text{atm})} = 0.60 \left(\frac{P_g}{V_R}\right)^{0.4} \left(v_S\right)^{0.5}(N(\text{rpm}))^{0.5} \]

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

\[ v_S = \frac{10^4 \text{ liters/min}}{\left(10^3 \text{ cm}^3/\text{liter}\right)} = 318.3 \text{ cm/min} \]

\[ k_{La} = 0.60(3.13)^{0.4}(318.3)^{0.5}(200)^{0.5} = 169 \text{ (mmole O}_2/(l \cdot \text{hr} \cdot \text{atm})} \]
Example Problem Solution (cont.)

\[
\text{OUR} = X \frac{dO_2}{dt} = \left( \frac{5 \text{ g cells}}{\text{liter}} \right) \left( \frac{20 \text{ mmoles O}_2}{\text{g cells/hr}} \right)
\]
\[
= 100 \frac{\text{mmoles O}_2}{\text{liter/hr}}
\]

\[
\text{OTR} = k \alpha (P_{O_2} - P^*)
\]

\[
P^* \text{ for } C_L = 1 \frac{\text{mg O}_2}{\text{liter}} = H_{O_2} C_L
\]
\[
= \left( \frac{0.21 \text{ atm}}{8 \frac{\text{mg O}_2}{\text{liter}}} \right) \left( \frac{1 \text{ mg O}_2}{\text{liter}} \right) = 0.0263 \text{ atm}
\]

Since \(\text{OUR} > \text{OTR}\), we must modify the bioreactor operation in order to bring them into balance

- \text{increase } N
- \text{use pure O}_2 \text{ rather than air.}
Measurement of OTR

\[
\frac{dC}{dt} = k_a (C^* - C_t) \\
 t = 0, C_t = 0 \\
\ln(C^* - C_t) = -(k_a t)
\]

Heat Generation Rate: Aerobic Growth

\[
Q_{GR} \approx 0.12 \text{ (OUR)} \\
\text{(} \frac{\text{kcal}}{\text{L} \cdot \text{hr}} \text{)} \\
\text{(} \frac{\text{mmol } Q}{\text{L} \cdot \text{hr}} \text{)}
\]
Heat Generation Rate: Agitation

\[ Q_{\text{aglt}} = \frac{P_g \text{ (power input aerated v\epsilon)}}{V_R \text{ (working volume of reactor)}} \approx \left(\frac{1 \text{ hp}}{100 \text{ gal}}\right) \]

Heat Balance

\[ HRR = \text{heat removal rate} = U A \Delta T_{\text{LM}} \]
\[ U = \text{overall heat transfer coefficient} \]
\[ A = \text{surface area of heat transfer surface} \]
\[ \Delta T_{\text{LM}} = \text{log mean temperature difference} \]
\[ = \frac{(T - t_2) - (T - t_1)}{\ln[(T - t_1)/(T - t_2)]} \]
\[ T = \text{bioreactor fluid temperature} \]
\[ t_1 = \text{cooling water inlet temperature} \]
\[ t_2 = \text{cooling water outlet temperature} \]