Comments on Other Bioreactor Considerations (Chapter 10)

Topics

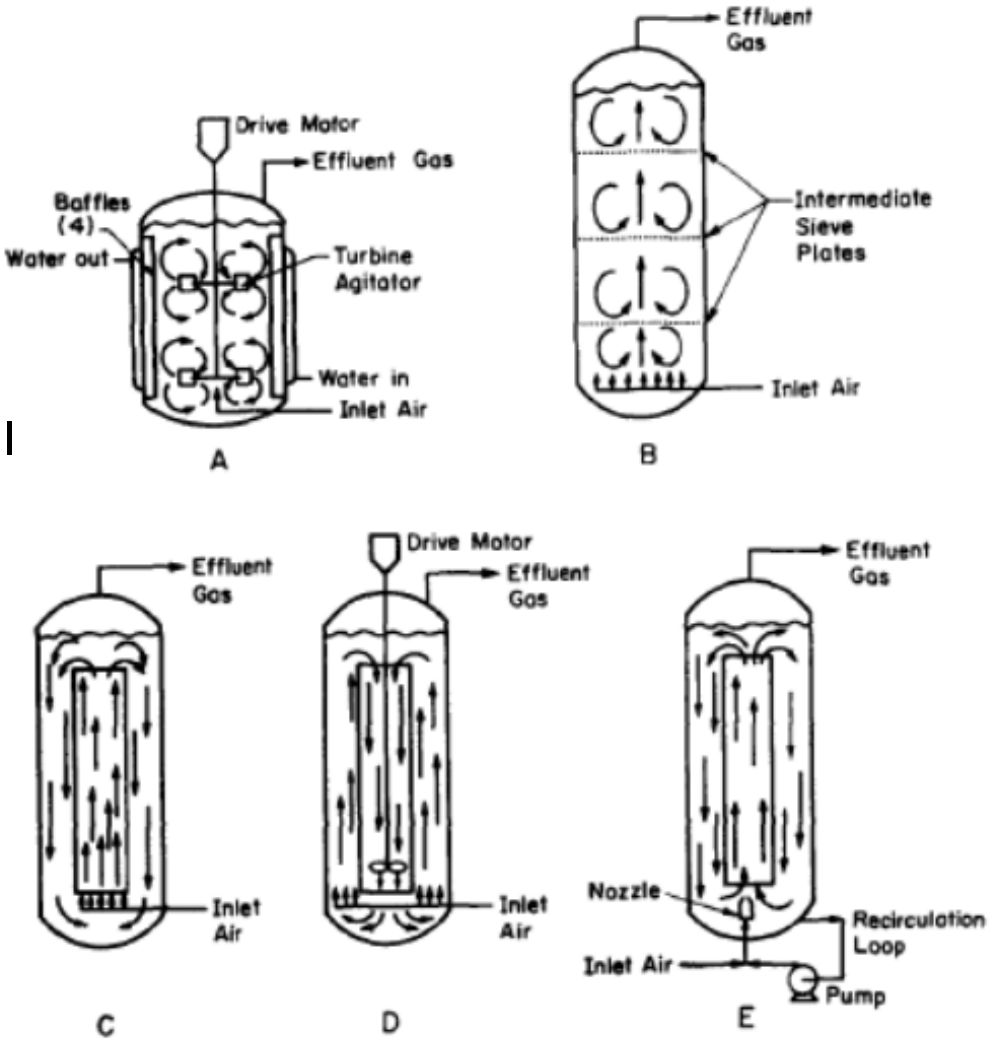
Concepts concerning non-laboratory reactors

- Agitation – mechanical, gas sparging, looping
- Scale up
- Sterilization

Agitation

Bioreactor types

- Stirred-tank reactor
- Bubble-column reactor,
- Airlift loop reactor with central draft tube
- Propeller loop reactor
- Jet loop reactor



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Aeration

Severity of oxygen requirements depends on type of organism

OUR = Oxygen Uptake Rate:

$$OUR = X \cdot q_{O2} = (k_L \cdot a)\,(C^* - C_L)$$

Typical correlation for the volumetric transfer coefficient $(k_L \cdot a)$:

$$(k_L \cdot a) = k\,(P_g/V_R)^{0.4}\,(v_s)^{0.5}\,(N)^{0.5}$$

where:
- P_g power requirement (e.g., kW)
- V_R reactor volume (e.g., L)
- v_s superficial gas exit speed (e.g., m/s)
- N rotational speed (e.g., rpm)

Values can be estimated or used for scale up / scale down

Aeration

Can measure ($k_L \cdot a$) from the N_2 stripping of O_2 from water

Dynamic relationship:

$$\frac{dC_L}{dt} = (k_L a)(C^* - C_L) \quad \Rightarrow \quad \frac{1}{(C^* - C_L)} \frac{d(C^* - C_L)}{dt} = -(k_L a)$$

$$\ln\left(\frac{C^* - C_L}{C^* - C_{L0}}\right) = -(k_L a)$$

Can get ($k_L \cdot a$) from slope of results on semi-log basis

Scale Up & Scale Down

Change in bioreactor size will change the environment experienced by the organisms. Scaling laws try to keep as much of the environment as similar as possible

Typical scaling parameters

- Volume: $V \propto D_o^3$ (if D_o/L held constant)
- Power input: $P \propto N^3 D_i^5$
- Impeller pump rate: $Q \propto N D_i^3$
- Energy per volume: $P/V \propto N^3 D_i^2$
- Pump rate per volume: $Q/V \propto N$

Other considerations

- Reynolds number: $N D_i^2 \rho / \mu$
- Shear @ impeller tip: $N D_i$

Scale Up & Scale Down

Time constants

- Flow: L/v or V/Q
- Diffusion: L^2/D
- Oxygen transfer: $1/k_La$
- Heat transfer: $V\rho C_p/UA$
- Mixing: $t_m = 4V/(\ 1.5\ N\ D^3\ )$ (stirred vessel)
  $t_m \propto V^{0.3}$ (0.1 – 100 m^3 fermenter)
- Conversion processes:
  - Growth: $1/\ \mu$
  - Chemical reaction: C/r
  - Substrate consumption: $C_S/r_{max}\ (\ C_S >> K_S)$
    $K_S/r_{max}\ (\ C_S << K_S)$
    Heat production: $\rho C_p\ \Delta_k T/r\Delta H$

Scale-Up Example

Scale up a 5 L bioreactor to 50 L

- 5 L reactor: $D_o/L = 1 \rightarrow D_o = 0.185$ m & $L = 0.185$ m
  $D_i/D_o = 0.4 \rightarrow D_i = 0.074$ m
  $N = 500$ rpm
- 50 L reactor: keep same D_o/L & D_i/D_o
  $D_o/L = 1 \rightarrow D_o = 0.399$ m & $L = 0.399$ m
  $D_i/D_o = 0.4 \rightarrow D_i = 0.160$ m

Calculate N to keep Re the same (essentially the product $N D_i^2$):

$N = (500 \text{ rpm})*(0.185/0.399)^2 = 108$ rpm

Calculate N to keep tip speed the same (essentially the product $N D_i$):

$N = (500 \text{ rpm})*(0.185/0.399) = 232$ rpm

Sterilization

Fluid streams sterilized…

- Physical removal of cells & viruses or…
- Inactivation of living particles by heat, radiation, and/or chemicals
  - Require understanding of kinetics of death

Require understanding of probabilistic nature of cell death

Sterilization – Probability of Extinction

Probability of extinction of the total population:

$$P_0(t) = \left[1 - p(t)\right]^{N_0}$$

where: p(t) probability that an individual will still be viable
N_0 number of individuals initially present

Expected number of individuals present at a time:

$$E\left[N_0(t)\right] = N_0 \cdot p(t)$$

Sterilization – Probability of Extinction

Specific death rate, k_d:

$$k_d = -\frac{1}{\left[N_0(t)\right]}\frac{d\left[N_0(t)\right]}{dt} \quad \Rightarrow \quad k_d = -\frac{1}{p(t)}\frac{d\left[p(t)\right]}{dt}$$

Simplest form for the probability function

$$p(t) = \exp\left(-k_d t\right)$$

May define the *decimal reduction time*, D, the time get a 10X reduction in the viable cells

$$0.1 = \exp\left(-k_d D\right) \quad \Rightarrow \quad D = -\frac{\ln(0.1)}{k_d} = \frac{2.303}{k_d}$$

Sterilization – Probability of Extinction

Specific death rate follows Arrhenius temperature expression

$$k_d = \alpha \exp\left(-\frac{E_{0d}}{RT}\right) \quad \Rightarrow \quad \ln\left(k_d\right) = \alpha' - \left(\frac{E_{0d}}{R}\right)\frac{1}{T}$$

Typical values for E_{od}

- Generally 50 – 150 kcal/g.mol
- *Bacillus stearothermophilus* ~70 kcal/g-mol
- *E. coli* ~127 kcal/g-mol

Spores have typical k_d values 0.5 – 5.0 min^{-1}

E_{od} vitamins & growth factors in many media 2 – 20 kcal/g-mol

- Need to completely kill foreign organisms …
- … without the destruction of growth factors in the medium

Sterilization – Probability of Extinction

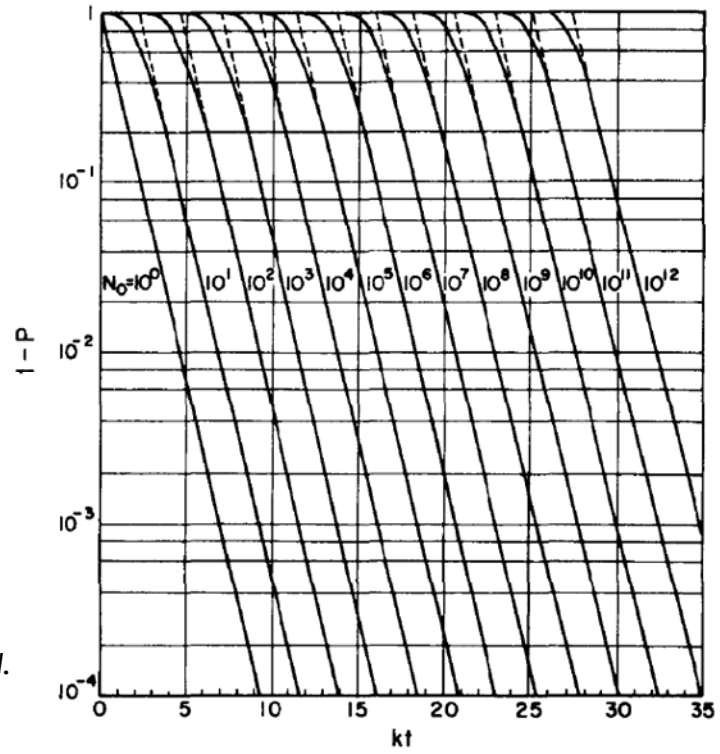
Probability of an <u>unsuccessful</u> fermentation:

$$1-P_0(t)=1-\left[1-p(t)\right]^{N_0} \quad \Rightarrow \quad 1-P_0(t)=1-\left[1-e^{-k_d t}\right]^{N_0}$$

N_0 is the number of individuals. Using n_0 (the concentration):

$$1-P_0(t)=1-\left[1-e^{-k_d t}\right]^{V\,n_0}$$

Leads to probability chart to relate number of organisms (volume times concentration) & the time of sterilization



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Sterilization – Probability of Extinction

Example of sterilization of bioreactor…

- for $k_d t = 15$ & $n_0 = 10^4$ spores/L…
- 1 L lab bioreactor unsuccessful sterilization:

$$1 - P_0(t) = 1 - \left[1 - e^{-k_d t}\right]^{V n_0} = 1 - \left[1 - e^{-15}\right]^{(1)(10^4)} = 0.003$$

- 10,000 L industrial bioreactor unsuccessful sterilization:

$$1 - P_0(t) = 1 - \left[1 - e^{-k_d t}\right]^{V n_0} = 1 - \left[1 - e^{-15}\right]^{(10000)(10^4)} = 1.0$$

- 10,000 L industrial bioreactor to get 0.003 unsuccessful sterilization:

$$0.003 = 1 - \left[1 - e^{-k_d t}\right]^{(10^4)(10^4)} \quad \Rightarrow \quad k_d t = -\ln\left[1 - (1 - 0.003)^{1/10^8}\right] = 24.2$$

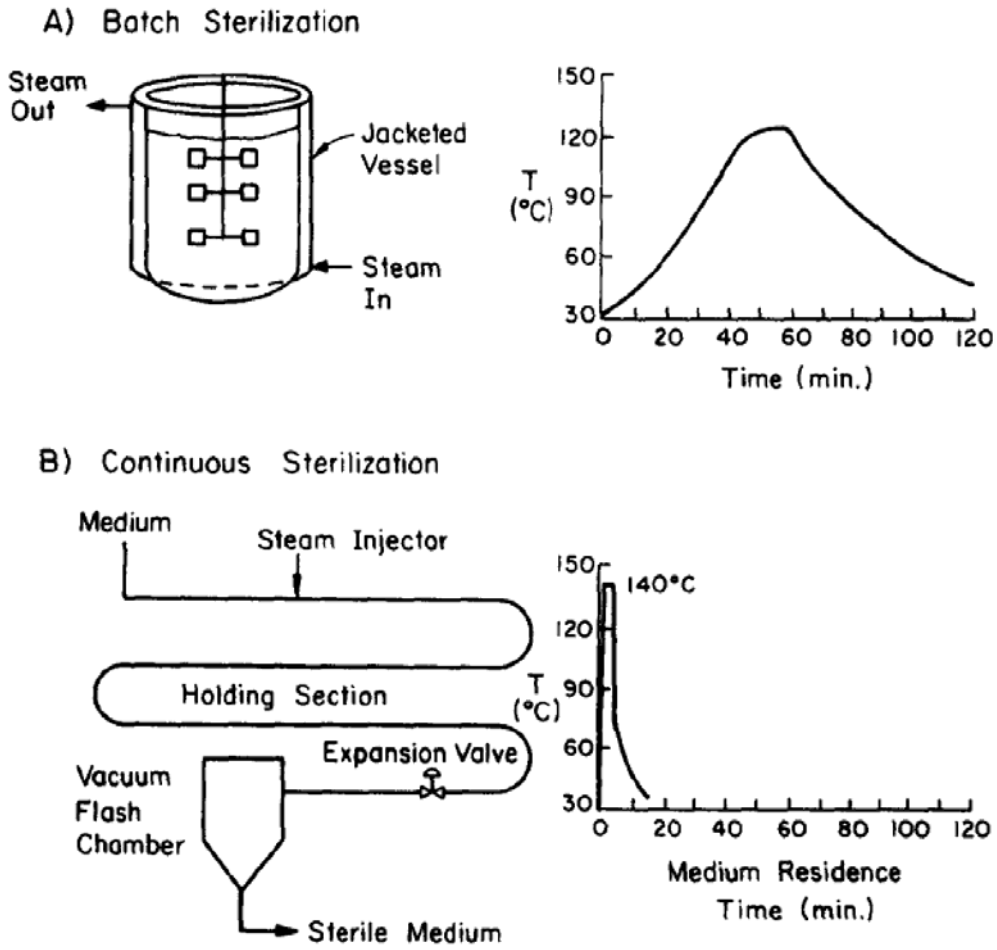
Sterilization

Factors in sterilization protocol: temperature, time of exposure, & initial number of organisms that must be killed

- Sterilization typically at 121°C

Direct injection of steam (continuous) typically requires a shorter residence time

- Potential disadvantages: dilution of medium, foaming



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Sterilization

Continuous sterilization example…

- 1,000 L/h feed with 100 spores/L to be sterilized so that there is only a 99% chance of contamination over a 1 week period. How long should the feed be held for continuous sterilization if $k_d = 1.2\ \text{min}^{-1}$?

$$N_0 = \left(100\ \frac{\text{spore}}{\text{L}}\right)\left(1000\ \frac{\text{L}}{\text{h}}\right)\left(24\ \frac{\text{h}}{\text{day}}\right)(7\ \text{day}) = 1.68\cdot 10^7\ \text{spores}$$

$$1 - P_0(t) = 1 - \left[1 - e^{-k_d t}\right]^{N_0} \quad \Rightarrow \quad t = -\frac{1}{k_d}\ln\left(1 - \left[P_0(t)\right]^{1/N_0}\right)$$

$$= -\frac{1}{1.2}\ln\left(1 - \left[0.99\right]^{1/1.68\cdot 10^7}\right) = 17.7\ \text{min}$$

- … 99.9% chance …

$$t = -\frac{1}{1.2}\ln\left(1 - \left[0.999\right]^{1/1.68\cdot 10^7}\right) = 19.6\ \text{min}$$

Summary

Agitation

Scale Up

Sterilization