MICROBIAL MODELING IN FOOD PROCESSING MODELS

BAEN-625 Advances in Food Engineering
Predictive Models

- Primary models
  - Growth models
  - Inactivation models
- Secondary models
- Tertiary models
Primary models

- They describe the microbial growth under isothermal conditions.
- The bacterial growth curve is sigmoid shape.
- There are four phases of bacterial growth.
  - Lag phase
  - Logarithmic or exponential growth phase
  - Stationary phase
  - Death phase
Sigmoidal (plus death) pattern of a typical microbial growth curve
Lag phase

- Bacteria adapt themselves to growth conditions.
- It is the period where the bacteria are maturing and not yet able to divide.
- During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.
Exponential phase

- It is a period characterized by cell doubling.
- The number of new bacteria appearing per unit time is proportional to the present population.
- If growth is not limited, doubling will continue at a constant rate.
Exponential phase

- Plotting the natural logarithm of cell number against time produces a straight line.
- The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time.
Exponential phase

- The actual rate of this growth (i.e. the slope of the line in the figure) depends upon the growth conditions.
- Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.
Stationary phase

- The growth rate slows as a result of nutrient depletion and accumulation of toxic products.
- This phase is reached as the bacteria begin to exhaust the resources that are available to them.
- This phase is a constant value as the rate of bacterial growth is equal to the rate of bacterial death.
Death phase

- Bacteria run out of nutrients and die
- The growth rate becomes negative.
- Food safety specialists are not usually interested in this death phase.
- Thus, only the first three phase are modeled.
Growth models

Because the bacterial growth curve is sigmoid in shape, several sigmoidal growth functions such as modified Gompertz model (1) and logistic model (2) to fit growth data are used:

1. \[ x(t) = x_o + (x_{max} - x_o) \exp[-\exp(-B(t - M))] \]

2. \[ x(t) = x_o + \frac{x_{max} - x_o}{1 + \exp(-B(t - M))} \]

\[ x(t) \rightarrow [\log(CFU / g)] \]

\[ x_o \rightarrow \text{initial concentration} [\log(CFU / g)] \]

\[ B \rightarrow \text{max relative growth rate at } M \text{ in } [1/h] \]

\[ M \rightarrow \text{time absolute growth is max in } [h] \]
Inactivation models

- Relationship between microbial population and time, when subject to a lethal treatment
- These models can follow a variety of patterns
Inactivation models

- A: tailing pattern
- B: increase in population, due to spore activation, before inactivation
- C: lag or shoulder before inactivation
- D: Sigmoidal pattern with a lag and a tail
Log-linear models

- Vast majority of inactivation data are presumed to follow a log-linear pattern with time.
- They are derived from mathematical analogy to 1st order reaction kinetics.

\[
\frac{dN}{dt} = -kN
\]

\[
\ln \left( \frac{N}{N_0} \right) = -kt
\]

- \(N\): Number of surviving bacteria
- \(k\): Inactivation rate constant [1/s]
- \(t\): Time [s]
$D_{10}$-value vs k

- Time required to achieve a 1-log (90%) reduction in the population

$$D_{10} = \frac{\ln(10)}{k}$$
$D_{10}$-value

$$\log\left(\frac{N}{N_o}\right) = \frac{t}{D_{10}}$$

$$D_{10} = \frac{1}{\text{slope}}$$
Weibull function

- Evidence shows that inactivation data are not linear
- Every cell in a microbial population has its own resistance to the lethal agent
- Resistance can be expressed as the time of exposure until the cell is no longer viable
- Weibull function – describes the distribution of resistance within a population
Weibull function

- Cumulative distribution of the Weibull function:

\[
\log \left( \frac{N}{N_o} \right) = -bt^n
\]

- \( b \): nonlinear rate parameter
- \( n \): shape factor
- \( n < 1 \) concave upward (A curve)
- \( n > 1 \) concave downward (C curve)
- \( n = 1 \) log-linear model
Secondary models

- Those that describe the effects of environmental conditions, such as temperature, pH, $a_w$, oxygen availability, added preservatives, etc. on the parameters of a primary model, particularly maximum growth rate.
Tertiary models

- They are defined as the integration of a primary and secondary models with a user-friendly interface.
- Two tertiary modeling tools are freely available and widely used in the United States—
  - AMI Process Lethality Spreadsheet (AMI-PLS; [http://www.amif.org](http://www.amif.org)), available from the American Meat Inst. and
  - Pathogen Modeling Program (PMP, v.7.0) developed by the USDA—Agricultural Research Service ([USDA 2003](http://www.usda.gov)).
Microorganism inactivation by irradiation is based on the assumption that all the cells or spores in a population have identical sensitivity to irradiation, and that it is the chance of an electron or photon striking the cell or target within the cell that will determine the death rate. According to this theory the destruction rate of microbial spores or cells at a given dose can be described by:

\[
S = \frac{N}{N_o} = e^{-D/D_o}
\]

\[
\ln S = -\frac{D}{D_o}
\]

\[
\log S = -\frac{D}{D_{10}}
\]

\[
D_o = \frac{D_{10}}{2.303}
\]

\[
S = \frac{N}{N_o} = 10^{-D/D_{10}}
\]

**N** = cell population  
**N₀** = initial cell population  
**S** = survival ratio \((N/N₀)\)  
0 < S < 1
Food Irradiation

- Most used is the log-linear model

\[
\log\left(\frac{N_o}{N}\right) = \frac{Dose}{D_{10}}
\]

\[
D_{10} = \frac{1}{\text{slope}}
\]
Log $S(N/No)$ vs Dose
Single-target, single-hit model

- The exponential behavior can be accounted for by a single-target, single-hit model of cell survival.

- The assumptions for the development of the single-hit inactivation model are
  - The deposition of energy as ionizing or excitation in the critical site leads to the production of molecular lesions in the cell and thus inactivation of the microorganisms and,
  - A cell will survive only if it has received no hits at all and that it will always die if it has received one or more hits.
Criticism of the linear model

- Target theory does not describe all experimental data
  - At low doses data show a shoulder, only show exponential response at higher doses
- The first-order kinetic does not account for the various possible mechanisms that are responsible for a vegetative cell demise or a spore ionizing radiation inactivation.
Multi-target, single-hit model

- Proposes 2 or more targets in a cell; each must receive a single hit before the cell is killed.

\[ S = 1 - \left(1 - e^{-D/D_o}\right)^n \]

- \(n\) is the extrapolation number
Multi-target, single-hit model

- It is useful for describing the response of cells at high doses
- Does not describe survival response at lower more doses
- Imply zero slope at very low doses; most data show finite or non-zero initial slope
Lethal, potentially Lethal Damage (LPL) Model

- Ionizing radiation produces 2 kinds of lesions:
  - repairable (potentially lethal)
  - lesions and non-repairable (lethal) lesions

- The non-repairable lesions produce single hit lethal events; linear component of cell kill.

- The effect of the repairable lesions depends on the competing processes of repair and binary mis-repair; leads to quadratic component.

- At higher doses the probability of binary interaction of potentially lethal lesions increases.
Lethal, potentially Lethal Damage (LPL) Model
Theoretical approach to calculate $D_{10}$-value

- **SCHEMATIC REPRESENTATION OF THE SIMULATED RADIATION PROCESS.**
- **THE BLUE DOTS INDICATE THE DETECTOR LOCATIONS.**
- **EACH INTERVAL ON THE X-, Y- AND Z- AXES CORRESPONDS TO THE LENGTH OF 50 NM, 20,000 NM, AND 20,000 NM, RESPECTIVELY.**
Theoretical approach to calculate $D_{10}$-value

- ILLUSTRATIONS OF DNA MODEL IN RANDOM ORIENTATION
- B form DNA created inside the detector
Theoretical approach to calculate $D_{10}$-value

$DSB_{nuc} = \frac{V_{nuc}}{V_{cell}} DSB_{con}$

$N_o = \frac{V_{con}}{V_{cell}}$

- double strand breaks in the container, $DSB_{con}$
- double strand breaks in the nucleoid, $DSB_{nuc}$
- $V_{cell} =$ the volume of a bacterial cell
- $V_{nuc} =$ the nucleoid volume in a single cell
Theoretical approach to calculate $D_{10}$-value

- Lethal probability

\[ P_{\text{lethal}} = \left( \frac{DSB_{\text{nuc}}}{G_{eq} N_0} \right)^{G_{eq}} \]

- $G_{eq}$ is the number of genome equivalents
- To completely inhibit the cell division, one or more DSB must be present in all of the existing genomes inside the cell.
- This equation accounts for the effect of having extra copies of the genetic material on the radiation resistance.
- Under the normal condition, the value of 3, which represents the average value of $G_{eq}$ of the microbial population, is assumed in the calculations.
Theoretical approach to calculate $D_{10}$-value

- **Survival probability**
  
  \[ P_{\text{survival}} = 1 - P_{\text{lethal}} \]

- At the *ith*-dose level, the number of DSB in the nucleoids is
  
  \[ DSB_{\text{nuc}}^i = DSB_{\text{nuc}}^{i-1} + DSB_{\text{nuc}}^1 \times P_{\text{survival}}^{i-1} \]

- As the container receives higher radiation dose, the chance that the lethal damage will occur in the already inactivated cells increases.

- The calculation of the survival probability is repeated for each linear dose increment until $DSB_{\text{nuc}}^i$ approaches the value of $G_{eq} \times N_0$. 
Effect of bacterial genome

The percentages of DNA lesion resulting from the direct effect, the damage ratios, and the calculated $D_{10}$ values for the cells with different genomes

<table>
<thead>
<tr>
<th>DNA sequence</th>
<th>% GC content</th>
<th>% DSB direct</th>
<th>% SSB direct</th>
<th>% BD direct</th>
<th>SSB DSB</th>
<th>BD DSB</th>
<th>BD SSB</th>
<th>$D_{10}$ (kGy)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli O157:H7</td>
<td>50.38</td>
<td>25.00</td>
<td>5.27</td>
<td>0.90</td>
<td>23.52</td>
<td>57.04</td>
<td>2.43</td>
<td>0.357 a</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>38.04</td>
<td>28.86</td>
<td>5.25</td>
<td>1.02</td>
<td>25.12</td>
<td>58.36</td>
<td>2.32</td>
<td>0.373 ab</td>
</tr>
<tr>
<td>AT</td>
<td>0.00</td>
<td>34.14</td>
<td>4.81</td>
<td>1.29</td>
<td>28.85</td>
<td>56.92</td>
<td>1.97</td>
<td>0.406 b</td>
</tr>
<tr>
<td>GC</td>
<td>100.00</td>
<td>22.28</td>
<td>5.72</td>
<td>0.91</td>
<td>20.86</td>
<td>59.29</td>
<td>2.84</td>
<td>0.340 a</td>
</tr>
</tbody>
</table>

* The $D_{10}$ values that do not share the same superscript letter are significantly different ($P < 0.05$).

- base damage, BD
- double strand break, DSB
- single strand break, SSB
Effect of bacterial genome

- The fractions of the DNA damage due to **direct effect**
  - Lesions generated by the interaction between the radiation and the DNA constitute only a small portion of the total damage

- The dominance of the **indirect effect** is most prominent in the case of BD
  - 99% of BD resulted from the reactions with water radicals
  - The degree of dominance becomes slightly smaller in the case of SSB
    - The two main radicals that can attack the nitrogenous base, the $e_{aq}$ does not produce significant damage to the sugar phosphate backbone
Effect of bacterial genome

- The electron impact ionization cross section of the sugar phosphate backbone is generally higher than that of the nitrogenous base.

- The contribution of the direct effect
  - Much greater in the induction of DSB (>22%) than in the production of SSB (<6%)
  - In the production BD (<2%)
SURVIVAL CURVES OF THE BACTERIAL CELLS CONTAINING DIFFERENT GENOMES
Effect of bacterial genome

- A 4.4% increase in the $D_{10}$ value was obtained when the genome of *L. monocytogenes* was used instead of the genome of *E. coli O157:H7*.

- Radiation sensitivity of a bacterium decreases with lesser GC content
The effect of $G_{eq}$ on the radiation sensitivity of the population of bacteria

- The quantity of the genetic material in the nucleoid region does not usually equal to that of one complete genome of the bacterium.
- It does not remain unchanged over an extended period of time under normal metabolic conditions.
- The parameter $G_{eq}$ was introduced so that the impact of the variation in the amount of genome can be assessed.
The effect of $G_{eq}$ on the radiation sensitivity of the population of *E. coli* O157:H7
The effect of $G_{eq}$:

- When $G_{eq}$ is greater than 1, the shoulder on the curve of survival probability appears.
- The higher value of $G_{eq}$ suggests that more radiation might be required to inhibit the cell proliferation.
- For the cell to become reproductively dead, at least one DSB must occur on each complete genome.
- The survival curves associated with different values of $G_{eq}$ show that the $D_{10}$ values, which were calculated from the linear portion of the semi-log plot, do not change with $G_{eq}$.
The effect of $G_{eq}$ on the radiation sensitivity of the population of $E. coli$ O157:H7

If the $D_{10}$ values were to be determined by using the entire survival curve, which includes the coordinates of the origin, the $D_{10}$ values would be greater for higher $G_{eq}$.
The effect of $G_{eq}$...

- In a regular food item at its natural state, typically, there is a mixture of cells at different phases of growth at any given moment.
- The mass of DNA contained in the cell varies as the cell progresses through the cell.
- Relevant information such as the nutrients in the medium and the temperature during the storage prior to radiation processing that can greatly influence the amount of DNA inside the nucleoid could be employed to generate the distribution of $G_{eq}$ for the calculation of the lethal probability.
Radiosensitivity of bacteria

- **Growth phase**
  - More susceptible to inactivation during the exponential phase

- **Temperature of food**
  - More resistant at $T < 0$ (less mobility of free radicals)

- **Moisture content**
  - More resistant at dry state (no radiolysis of water)
Spores

- Hydrogen sulfide reduces effect of X-rays in dry spores by about 50% when given after irradiation,
  - removal of radicals that are toxic when combined with oxygen
- and by approximately 75 percent when present during irradiation.
  - the removal of radicals that become toxic in the absence of oxygen.