Factors Influencing the Growth of Microorganisms in Foods

PubH 7213

Applications of Microbiology to Food Systems Monitoring

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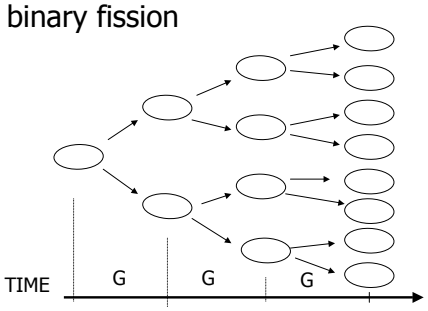
What is microbial growth?

Microbial growth

What is growth?

- Production of more living cells
- Reproduction
  - Plants and animals - sexual
  - Fungi - sexual and asexual
  - Bacteria – asexual
- Production of compounds
  - Flavors, polymers, others

Microbial growth: division or binary fission



Microbial growth: division or binary fission

$$N = N_o\, 2^{(t-t_o)/G} \qquad \text{Equation 1}$$

t_o = initial time
t = final time
N_o = initial number of organisms, cells or bacteria
N = final number of cells after time (t) elapsed
G = time to divide into two cells

Microbial growth or division

G= generation time = time to double

- Depending on nutrients, temperature, bacteria species, pH, the generation time can be as low as 20 minutes to several days.
- Microbial growth is an exponential function

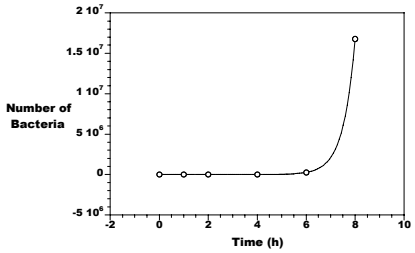
Microbial growth

$N = N_o\ 2^{(t-t_o)/G}$

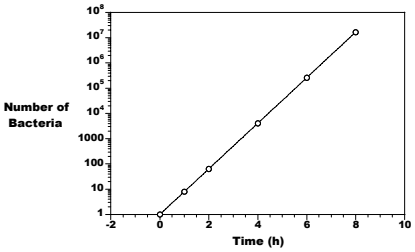
Example: G=20 min and N_o=1 cell

t value	
1 h	$N= 1 \times 2^{(60/20)} = 1 \times 2^3 = 8$ cells
2 h	$N= 1 \times 2^{(120/20)} = 1 \times 2^6 = 64$ cells
4 h	$N= 1 \times 2^{(240/20)} = 1 \times 2^{12} = 4{,}096$ cells
6 h	$N= 1 \times 2^{(360/20)} = 1 \times 2^{18} = 262{,}144$ cells
8 h	$N= 1 \times 2^{(480/20)} = 1 \times 2^{24} = 16{,}777{,}216$ cells

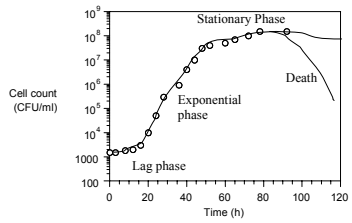
Microbial growth



Microbial growth



Typical Microbial Growth Curve



Growth of *Pseudomonas fragi* in milk at 10°C

Exponential growth: First rate kinetics

$$\frac{dN}{dt} = \mu N \qquad \text{Equation 2}$$

t = time

N = number of organisms, cells or bacteria

μ = specific growth rate (h^{-1})

Microbial growth: Specific Growth Rate (μ)

Integrating Eq. 1

$$\ln \frac{N_t}{N_o} = \mu(t - t_o) \qquad \text{Equation 3 (Monod)}$$

t_o = initial time

t = final time

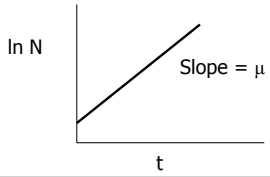
N_o = initial number of organisms, cells or bacteria

N_t = final number of cells after time ($t-t_o$) elapsed

Microbial growth: Specific Growth Rate (μ)

Solving for μ:

$$\mu = \frac{\ln N_t - \ln N_o}{t - t_o} \quad \text{Equation 4}$$



Microbial growth: Specific Growth Rate and Doubling Time (μ)

Find the mathematical relationship between μ and G

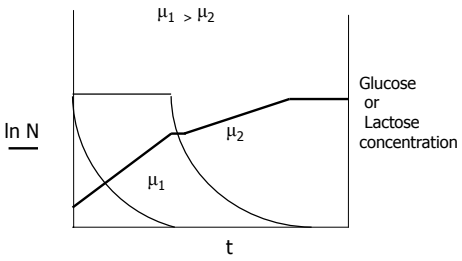
Microbial growth: Other mathematical models

- Gompertz equation
- Logistic model
- Baranyi model

Microbial growth: concepts

- Optimum growth: largest μ and smallest G
- Diauxic growth: changes in growth rate due to changes in substrate

Microbial growth: Diauxic growth



Microbial Growth: Mixed Populations

- Symbiotic growth
- Synergistic growth
- Antagonistic growth

Microbial Growth

- How do we measure growth?

Factors Influencing Microbial Growth in Foods

Factors that affect microbial growth

- Intrinsic: factors inherent to the food
- Extrinsic: storage conditions of the food

Parameters that affect microbial growth

- Intrinsic:
  1. pH
  2. Water activity (a_w)
  3. Oxidation-reduction potential
  4. Nutrient content
  5. Presence of antimicrobials
  6. Biological structures

Intrinsic factors that affect microbial growth

1. pH

What is pH? Acidity-alkalinity

$pH = - \log[H^+]$

- pH Scale

very acid neutral very alkaline

1- 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9- 10- 11- 12- 13-14

Acidic Foods | Most Foods | Eggs, tortillas

Intrinsic factors that affect microbial growth

1. pH

Optimum

Most pathogens cannot grow at pH <4.0

Some acid foods ≈ 5.0

Intrinsic factors that affect microbial growth

pH of selected foods

Food	pH	Food	pH
Milk	6.1 – 6.4	Beef	5.1 – 6.2
Cheddar cheese	5.9	Ham	5.9 – 6.1
Buttermilk	4.5	Chicken	6.2 – 6.4
Apples	2.9 – 3.3	Corn	7.3
Tomatoes	4.2 – 4.3	Lettuce	6.0
Limes	1.8 – 2.0		

Intrinsic factors that affect microbial growth

pH range of microorganisms

Microorganism	pH range
Gram positive bacteria	4.0 – 8.5
Gram negative bacteria	4.5 – 9.0
Molds	1.5 – 9.0
Yeasts	2.0 – 8.5

Intrinsic factors that affect microbial growth

1. pH
   Technologies for control
   1. Addition of acids
      - Vinegar to mayonnaise, relish,etc.
      - Citric acid to jams and jellies
      - Lactic acid - dairy
   2. Fermented foods
      - Yogurt, cheese
      - Pickles, vinegar

Intrinsic factors that affect microbial growth

Growth inhibition at low pH

Why bacteria cannot grow at acid pH?

Intrinsic factors that affect microbial growth

Growth inhibition at low pH

Principle:

Not all acids are the same

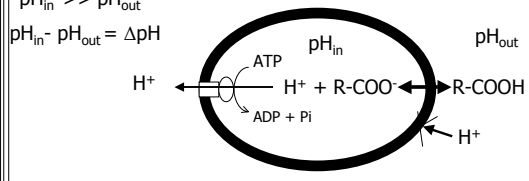
Why???

Intrinsic factors that affect microbial growth

Effects of organic acids on cells

$pH_{in} >> pH_{out}$

$pH_{in} - pH_{out} = \Delta pH$



Diffusivity and antimicrobial activity
Acetic>Propionic>>Lactic>>>>Citric>Malic

Intrinsic factors that affect microbial growth

2. Water activity (a_w) or %RH/100

$$a_w = \frac{P}{P_w}$$

- $a_w \neq$ moisture content
- a_w is a measure of the water "available" for microorganisms to grow or reactions to take place
- a_w of water = 1.00

Intrinsic factors that affect microbial growth

2. Water activity

	Minimum a_w
Bacteria	0.89-0.98
Yeast	0.7-0.88
Molds	0.6-0.85
S. aureus	0.85
C. botulinum	0.93
Salmonella	0.96

- no growth of any microbe below 60% RH ($a_w = 0.6$)

Intrinsic factors that affect microbial growth

Typical a_w of some foods

Food	a_w
Fresh fruits and vegetables	≥0.98
Fresh meats, fish	≥0.98
Cooked meat, bread	0.91-0.98
Cured meat, cheese	0.91-0.95
Syrups	0.87-0.91
Jams	0.75-0.80
Candies	0.65-0.75
Flour, milk powder	0.20-0.60

Intrinsic factors that affect microbial growth

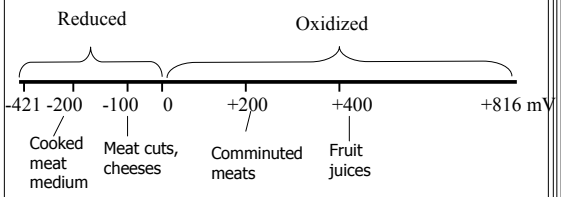
2. Water activity

Technologies to control a_w

a) Drying - cereals
b) Concentrate - preserves
c) Add salt - butter
d) Add sugars - icing
e) Glycols - pet foods

Intrinsic factors that affect microbial growth

3. Oxidation-Reduction potential (O/R, Eh)



Intrinsic factors that affect microbial growth

3. O/R potential or oxygen availability

Conditions that influence the food O/R

– Food composition – presence of SH groups

– Contact of food with atmosphere- packaging

Intrinsic factors that affect microbial growth

3. O/R potential or oxygen availability

Types of organisms

1. Aerobes – Require oxygen (+Eh)
2. Facultative anaerobes – Tolerate or use oxygen (+/-Eh)
3. Strict anaerobes – Require –Eh
   - Oxygen is toxic

Intrinsic factors that affect microbial growth

3. O/R potential or oxygen availability

Examples of food-borne microorganisms?

1. Aerobes
2. Facultative anaerobes
3. Strict anaerobes

Intrinsic factors that affect microbial growth

3. O/R potential or oxygen availability

Technologies to control O/R in foods

- Vacuum packaging
- Skin tight packaging
- Gas flushing
- Canning
- Antioxidants

Intrinsic factors that affect microbial growth

4. Nutrient content

Microorganisms require:

- Energy source
  - Carbohydrates, amino acids, proteins, organic acids
- Carbon source
- Nitrogen source
- Minerals

And may also need:

- Vitamins and other growth factors

Intrinsic factors that affect microbial growth

4. Nutrient content

What are the preferred energy source for most microorganisms?

Intrinsic factors that affect microbial growth

5. Presence of antimicrobials

- Natural constituents
  - Lysozyme – eggs
  - Lactoferrin – milk
  - Essential oils – spices and vegetables
  - Lactoperoxidase system
- Preservatives
  - Benzoic acid
  - Sorbic acid
  - Nisin

Intrinsic factors that affect microbial growth

6. Biological structures

- Natural physical barriers
  - Cell walls
  - Shells
  - Skin

Intrinsic factors that affect microbial growth

7. Presence and activities of other microorganisms

- Bacterial interference
- Competitive exclusion
  - Production of lactic acid
    - Fermented foods
  - Production of H_2O_2
  - Production of bacteriocins

Parameters that affect microbial growth

- Intrinsic: factors inherent to the food
- Extrinsic: storage conditions of the food

Parameters that affect microbial growth

- Extrinsic:

1. Temperature
2. Relative humidity of environment
3. Presence and concentration of gases
4. Presence and activities of other microorganisms

Extrinsic factors that affect microbial growth

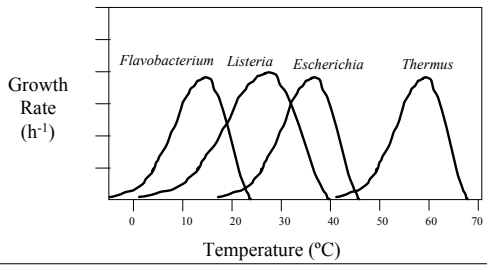
1. Temperature

Classification of microorganisms based on optimal growth temperature:

	Optimal	Overall range
Psychrophile	10 - 15 °C	-5 - 20 °C
Psychrotroph	20 - 30 °C	0 - 45 °C
Mesophile	30 - 40 °C	20 - 45 °C
Thermophile	55 - 65 °C	45 - 70 °C

Extrinsic factors that affect microbial growth

1. Temperature



Extrinsic factors that affect microbial growth

1. Temperature

Dependence of growth rate on T

$\mu = Ae^{-E_a/RT}$ Arrhenius equation

Integrating:

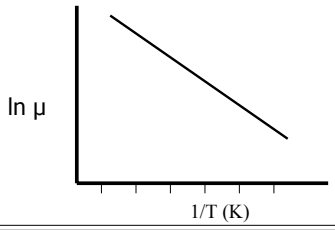
$\ln \mu = (-\Delta Ea/R)(1/T) + \ln A$

R = gas constant T= growth temperature
A= arrhenius constant E_a = activation energy

Extrinsic factors that affect microbial growth

1. Temperature

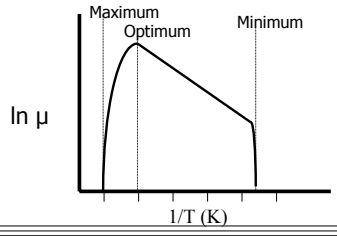
$\ln \mu = (-\Delta Ea/R)(1/T) + \ln A$



Extrinsic factors that affect microbial growth

1. Temperature

$\ln \mu = (-\Delta Ea/R)(1/T) + \ln A$



Extrinsic factors that affect microbial growth

1. Temperature

Minimum growth temperatures

Microorganism	Temp (°C)
Vibrio spp.	-5
Yersinia enterocolitica	-2
Some coliforms	-2
Aeromonas hydrophila	-0.5
Listeria monocytogenes	1.0
Clostridium botulinum	3.3
Salmonella panama	4.0
Vibrio parahemolyticus	5.0
Staphylococcus aureus	7.0

Extrinsic factors that affect microbial growth

1. Temperature

Growth Control Technologies:

- Refrigeration 32 to 40 °F
  - GMP's (CFR110.80b) < 45 °F
  - Many commercial systems > 42 °F

Problem: handling at high temperatures

Extrinsic factors that affect microbial growth

1. Temperature

Growth Control Technologies:

- Freezing: Ideal < 5 °F
  - GMP's (CFR110.80b) "frozen foods should be kept frozen"
  - It does not kill most pathogens

Extrinsic factors that affect microbial growth

2. Relative humidity

Atmospheric %RH will affect a_w and microbial growth

Control:
- Packaging
- Modified atmospheres

Extrinsic factors that affect microbial growth

3. Presence and concentration of gases
- CO_2, Ozone – antimicrobial properties

Control:
- Packaging
- Modified atmospheres

Why is so important to control microbial growth?

- Keep the numbers below the infectious dose
- Extend shelf-life

Key to Control of Microorganisms

Management of numbers

Microbial ecology-relationship between organisms and their environment, resulting in growth, survival, or death

Environment influences:
- Abiotic (physical/chemical)
- Biotic (other microbes & host)

Control Measures

- Four Basic Activities:
  - Selecting ingredients
  - Preventing contamination
  - Destroying pathogens
  - Preventing growth of pathogens

Operations aimed to kill microorganisms: "KILL STEP"

- Pasteurization
- Canning or commercial sterilization
- Cooking
- Other heat treatments
- Irradiation
- High-pressure treatment
- Fumigation