

Food Microbiology

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Factors Influencing Growth of Microorganisms in Food

- Understanding factors that influence microbial growth essential to maintaining food quality
 - In production and preservation
- Conditions naturally present in food termed **intrinsic factors**
- Environmental conditions are termed **extrinsic factors**
- Factors combine to determine which **microbes grow in particular food and at what rate**

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(a)



(b)



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Factors Influencing Growth of Microorganisms in Food

- **Intrinsic factors**
 - Multiplication of food greatly influenced by inherent characteristics of food
 - Microbes multiply most rapidly in **moist, nutritionally rich, pH neutral** foods
 - **Intrinsic factors include :**
 - Water availability
 - pH
 - Nutrients
 - Biological barriers
 - Antimicrobial chemicals

Factors Influencing Growth of Microorganisms in Food

- **Intrinsic factors**

- **Water availability**

- All microorganisms require water in an available form to grow and metabolize
- Availability of water is measured by **water activity (a_w)**
- $a_w = \text{Vapor pressure of food substrate} / \text{Vapor pressure of pure water at the same temperature}$
- a_w of pure water is 1.0
- % moisture of foods is not the same as a_w

- Foods vary dramatically in terms of water availability
 - Fresh meats and milk have high water content
 - Supports microbial growth
 - Breads, nuts and dried foods have low water availability
 - Defined populations can grow in these specific environments
- **Water activity** (a_w) used to designate amount of water available in foods
 - Pure water has a_w of 1.0
 - Most bacteria require a_w of above 0.90
 - Most fungi require a_w of above 0.80

- The a_w of a food can be reduced by increasing the concentration of solutes in the aqueous phase of the food
- Accomplished by drying, freezing, or addition of solutes (sugar in jams)
- All microorganisms have optimum and minimum a_w requirements
- The maximum limit is slightly less than 1.0 (organisms cannot grow in absolutely pure water)
- Yeasts and molds can tolerate lower a_w than bacteria
- Gram-negative bacteria require higher a_w than Gram-positive bacteria

- a_w affects growth, toxin production, spore germination, and heat resistance of microorganisms
- As a_w decreases, heat resistance increases
- Microorganisms will not grow in foods with a_w below 0.60
- Bacterial pathogens are usually inhibited at a_w less than 0.9 except *Staphylococcus aureus* can grow at a_w 0.86
- Most fresh foods have very high a_w (0.98-0.99), processed foods range (pudding 0.99; crackers 0.3)

Minimum Water Activity that will support the growth of bacteria and yeasts and molds

Microorganisms	Minimum a_w
Spoilage Bacteria	0.90-0.91
Spoilage Yeast	0.87-0.94
Spoilage Molds	0.70-0.80
Xerophilic molds	As low as 0.6
Clostridium botulinum	0.90-0.98
Salmonella spp	0.95-0.96
Staphylococcus aureus	0.86-0.92

FOOD PRESERVATION

- Most foods have a water activity above 0.95 and that will provide sufficient moisture to support the growth of bacteria, yeasts, and mold.
- The amount of available moisture can be reduced to a point that will inhibit the growth of microorganisms



Food	Water activity
Fresh meat and fish	0.99
Liverwurst	0.96
Cheese spread	0.95
Bread	0.95
Red bean paste	0.93
Caviar	0.92
Aged cheddar	0.85
Fudge sauce	0.83
Salami	0.82
Soy sauce	0.8
Jams and jellies	0.8
Peanut butter	0.7
Dried fruit	0.6
Cookies	0.3
Instant coffee	0.2

- **High Moisture Food**
- **Intermediet Moisture Food**
- **Low Moisture Food**

FOOD PRESERVATION

- **Reduced water availability**
 - Drying
 - Freeze-drying (lyophilization)
 - Addition of high concentrations of solutes such as sugar or salt



Food Preservation

- **Freezing**

- Stops microbial growth
 - **Water unavailable** due to ice formation
- Portion of organisms remaining can grow when food is thawed

- **Drying/reducing water availability**

- Inhibits microbial growth by decreasing available moisture
 - Molds may grow eventually

Effect of a_w on Spoilage of Foods

a_w	Spoilage microorganism	Food
0.90-1.00	Bacteria	Cottage cheese, meat
0.85 – 0.90	Bacteria, molds, yeasts	Margarine, condensed milk, whipped butter
0.80 - 0.85	Yeasts	Fruit syrups
0.75 - 0.80	Xerophilic molds, molds and yeasts	Dried figs, jams
0.70 - 0.75	Yeasts	Confections
0.65 - 0.70	Osmophilic yeasts	Molasses
0.60 - 0.65	Xerophilic molds, osmophilic yeasts	Dried fruit

Factors Influencing Growth of Microorganisms in Food

- Extrinsic factors
 - Extent of microbial growth largely dependent on storage of food
 - Microbes multiply rapidly in warm, oxygen-rich environments
 - Extrinsic factors include
 - Storage temperature
 - Atmosphere

Factors Influencing Growth of Microorganisms in Food

- **Extrinsic factors**

- Atmosphere

- **Presence or absence of oxygen** affects type of microbial population

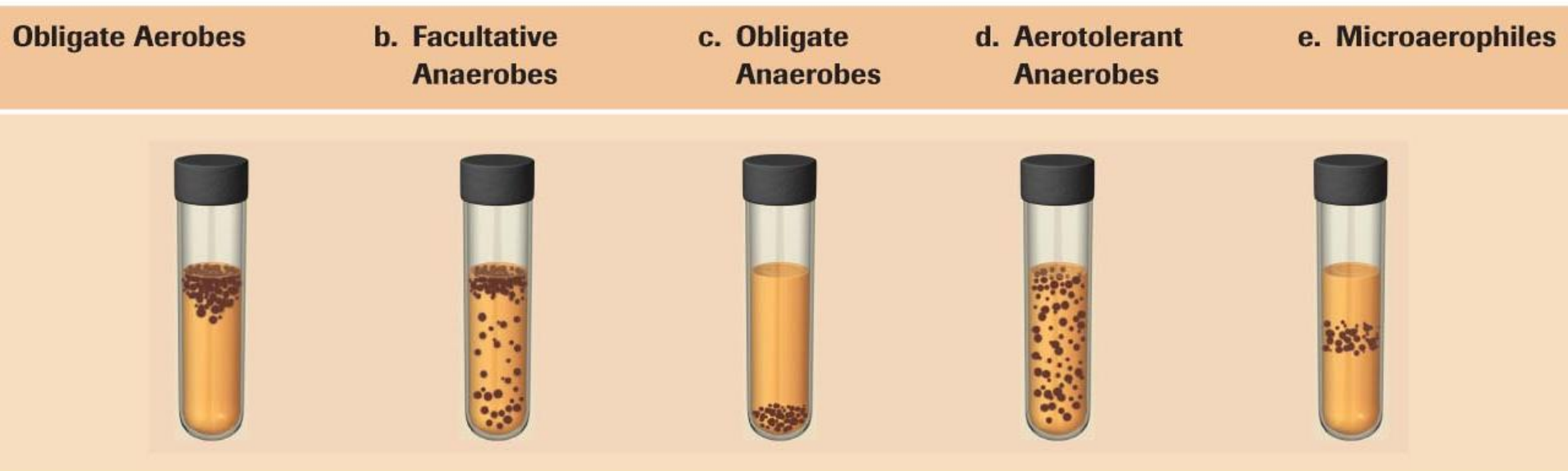
- Obligate aerobes cannot grow under anaerobic conditions

- Obligate anaerobes will grow in anaerobic conditions

- Including certain foodborne pathogens

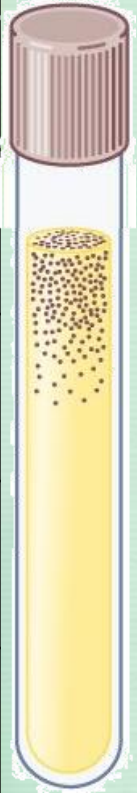
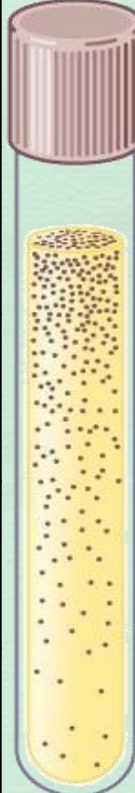

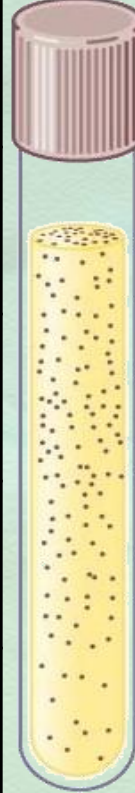
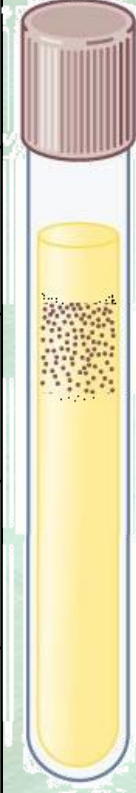
Chemical Requirements for Growth: **Oxygen**

O₂ requirements vary greatly



The Effects of Oxygen on the Growth of Various Types of Bacteria

Organism	Obligate aerobe	Facultative anaerobe	Obligate anaerobe	Aerotolerant anaerobe	Microaerophile
Effect of oxygen on growth	Oxygen required. Only can survive in aerobic conditions. Dies if oxygen is absent.	↑growth in presence of oxygen. Both aerobic and anaerobic	Oxygen not required. Only can survive in anaerobic conditions. Dies if oxygen is present.	Do not care if oxygen is present or absent. Just do not use the oxygen present	Low oxygen concentration allowed for growth only.
Enzymes effect on oxygen	CATALASE & superoxide dismutase (SOD) neutralise the toxic forms of oxygen		NO enzymes available to neutralize toxic oxygen	SOD present to partially neutralize oxygen	
Examples	<i>Mycobacterium</i>	<i>Streptococcus</i> , <i>Staphylococcus</i> , <i>Enterobacteriaceae</i>	<i>Clostridium</i>	<i>Lactobacillus</i>	<i>Neisseria gonorrhoeae</i>

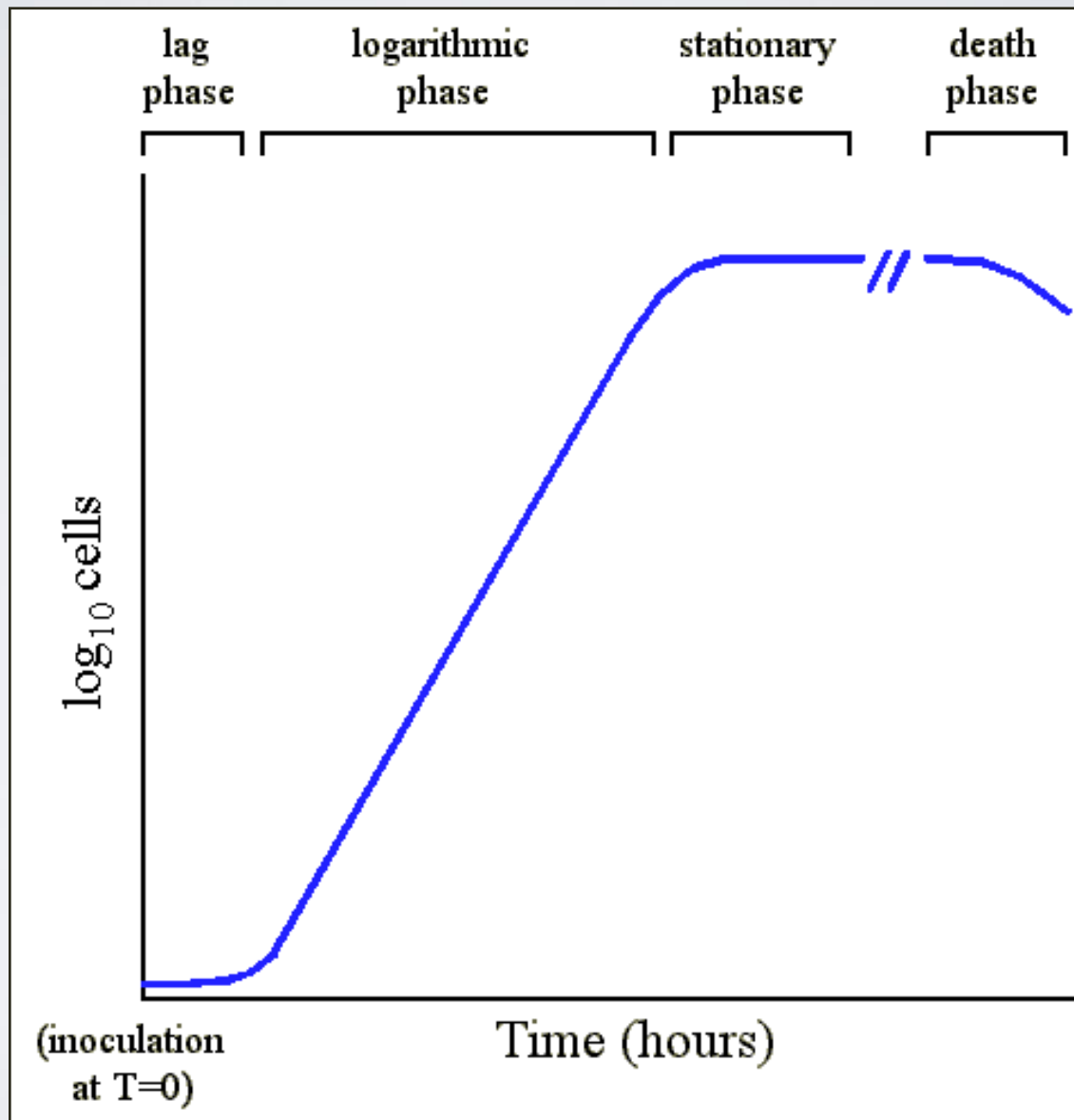
class Aspects	Obligate aerobes	Facultative anaerobes	Obligate anaerobes	Aerotolera - nt anaerobes	Microaerop -hiles
Energy source	cellular respiration	respiration, fermentation	fermentation , autotrophy	fermentation	cellular respiration
aerobic	✓ 	✓ 	✗ 	✗ 	✓ 
anaerobic	✗	✓	✓	✓	✗
SOD enzyme	✓	✓	✗	?	✓
Peroxidase	✓	✓	✗	✗	✓
catalase	✓	✓	✗	✗	✓
example	Bacillus, Pseudomonas	E. coli, Staphylococcus	Clostridium tetani, Bacteroides	Lactobacillus	Campylobacter & Helicobacter pylori

Factors that influence microbial activity

- A knowledge of the factors that **favor** or **inhibit** the growth of the microorganisms is important in understanding the principles of food spoilage and preservation.
- Six major factors:
 1. Moisture
 2. Oxygen
 3. Temperature
 4. Acidity, pH
 5. Nutrients
 6. Growth inhibitors



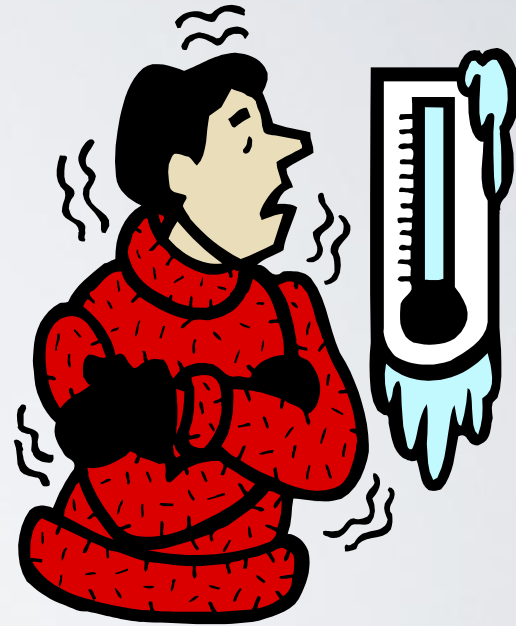
Bacterial Growth Curve



Temperature

Temperature has a profound effect on microorganism viability, primarily because enzyme-catalyzed reactions are sensitive to temperature

- At low temperatures, a temperature rise increases the growth rate by increasing the rate of enzyme reactions



- At high temperatures, microorganisms are damaged by enzyme denaturation, membrane disruption, and other phenomena
- Organisms exhibit distinct cardinal temperatures (minimal, maximal, and optimal growth temperatures)



Effect of Temperature

- Effect of temperature on proteins – can denature proteins at higher temperatures
- Effect of temperature on lipid-containing membranes of cells and organelles
 - If too low, membranes become rigid and fragile
 - If too high, membranes become too fluid and cannot contain the cell or organelle
- Therefore, temperature has an affect on the growth and survival of microbes

- Dependence of growth of microorganisms on temperature is largely related to activation and inactivation of enzyme systems in microorganisms.
- Three different effects of temperature contribute to the death of microbial cells:
 1. Denaturation of proteins (enzymes) by heat
 2. Intoxication due to accelerated metabolic reactions
 3. Changes in essential lipids. Melting points of the fats found in the organisms and temperature ranges of death are related.

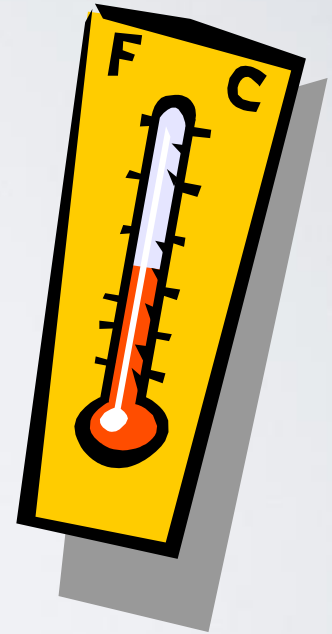
Effect of temperature

- Low temperature
 - Enzymatic reactions too slow; enzymes too stiff
 - Lipid membranes no longer fluid
- High temperature
 - Enzymes denature, lose shape and stop functioning
 - Lipid membranes get too fluid, leak
 - DNA denatures
- As temperature increases, reactions and growth rate speed up; at max, critical enzymes denature.

Effect of temperature on microbial growth

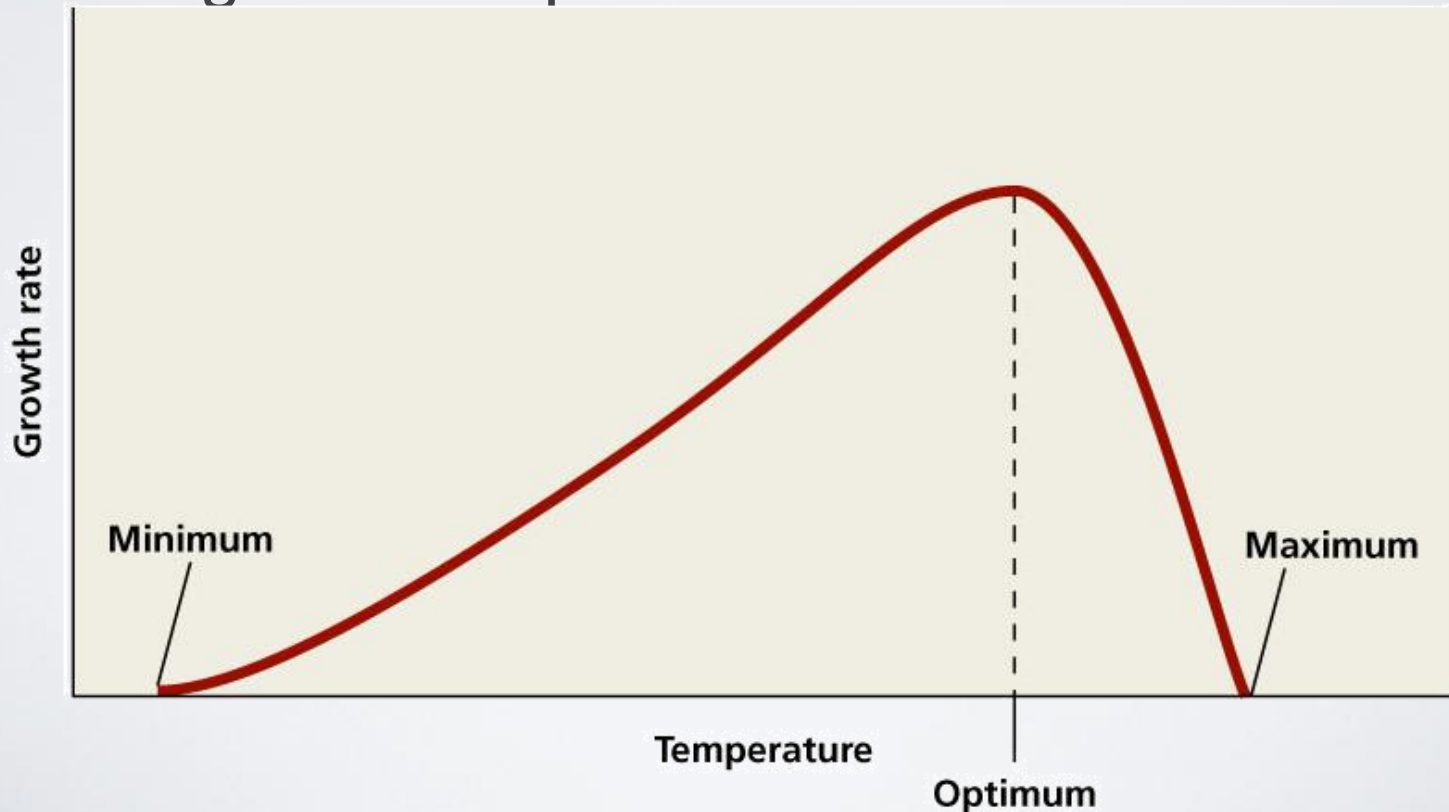
- Optimum, minimum and maximum temperatures for microbial growth.
- min. T. : refrigeration, freezing
- max. T. : heat processing

- Microorganisms are classified w.r.t. their temperature requirements.



Effects of Temperature on Growth

- Minimum, optimum and maximum temperature as determined by the graph showing growth rate plotted against temperature



Temperature

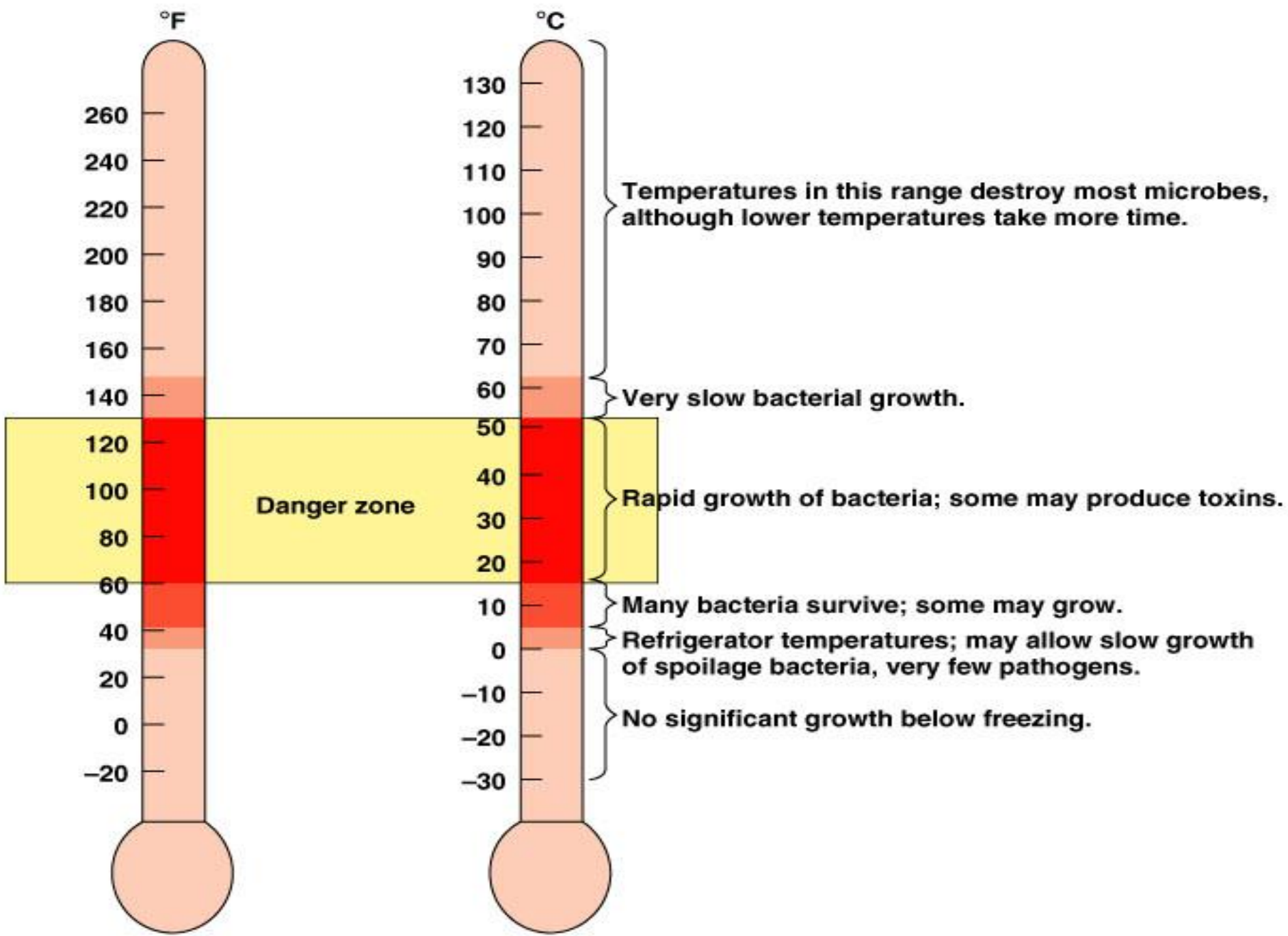
- The lowest temperature at which an organism is able to conduct metabolism is called ***minimum growth temperature***
 - Many bacteria will survive below this temperature, despite the fact the cell membrane is less fluid and transport processes are too slow
- The highest temperature at which an organism continues to conduct metabolism is the ***maximum growth temperature***
 - Above this value proteins denature and the cell dies

Optimum Growth Temperature

- Based on the *preferred temperature ranges* – the temperature at which their metabolic activity and growth are best supported – microbes can be classified into four overlapping groups

Bacteria and temperature

- Bacteria have temperature ranges (grow between 2 temperature extremes), and an optimal growth temperature. Both are used to classify bacteria.
- As temperature increases, so do metabolic rates.
- At high end of range, critical enzymes begin to denature, work slower. Growth rate drops off rapidly with small increase in temperature.



Terms related to temperature

- Special cases:
 - Psychrotrophs: bacteria that grow at “normal” temperature ranges (e.g. room temperature” but can also grow in the refrigerator; responsible for food spoilage.
 - Thermoduric: more to do with survival than growth; bacteria that can withstand brief heat treatments.

Optimum Growth Temperature

- *Psychrophiles* grow best at temperatures below 15 C and can grow at temperatures below 0 C
- They generally die above 20 C
- Include algae, fungi and bacteria that live in snowfields, ice and cold water
- Why do they not cause disease in humans?
- Can cause some food spoilage
- Hard to manage in laboratory



Optimum Growth Temperature

- **Mesophiles** are organisms that grow best at temperatures ranging from 20C to about 40C, but can survive at higher and lower temperatures
- Because body temperature is 37C human pathogens are mesophiles
- **Thermotolerant mesophiles** are mesophiles that can survive brief periods at higher temperatures
- Inadequate heating during pasteurization and canning can result in food spoilage by thermotolerant mesophiles

Optimum Growth Temperature

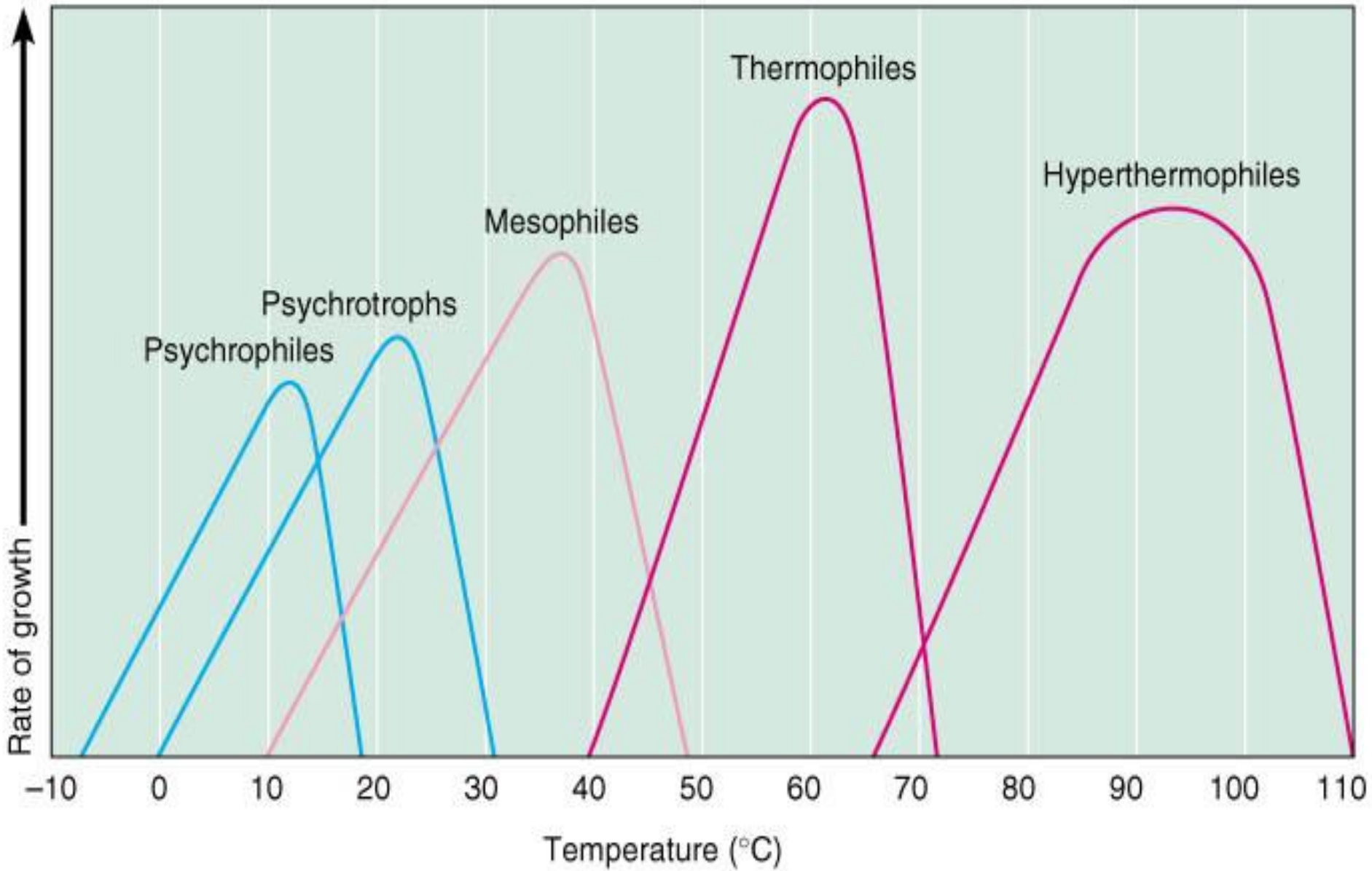
- Thermophiles grow at temperatures above 45C in habitats such as hot springs and compost piles
- Hyperthermophiles are archaea that can live in water above 80C. Others live above 100C
- Thermophiles and hyperthermophiles do not cause disease because they “freeze” at body temperature

Bacteria Classification

- Psychrophiles can grow well at 0°C, have optimal growth at 15°C or lower, and usually will not grow above 20°C
- Psychrotrophs (facultative psychrophiles) can also grow at 0°C, but have growth optima between 20°C and 30°C, and growth maxima at about 35°C
- Mesophiles have growth minima of 15 to 20°C, optima of 20 to 45°C, and maxima of about 45°C or lower

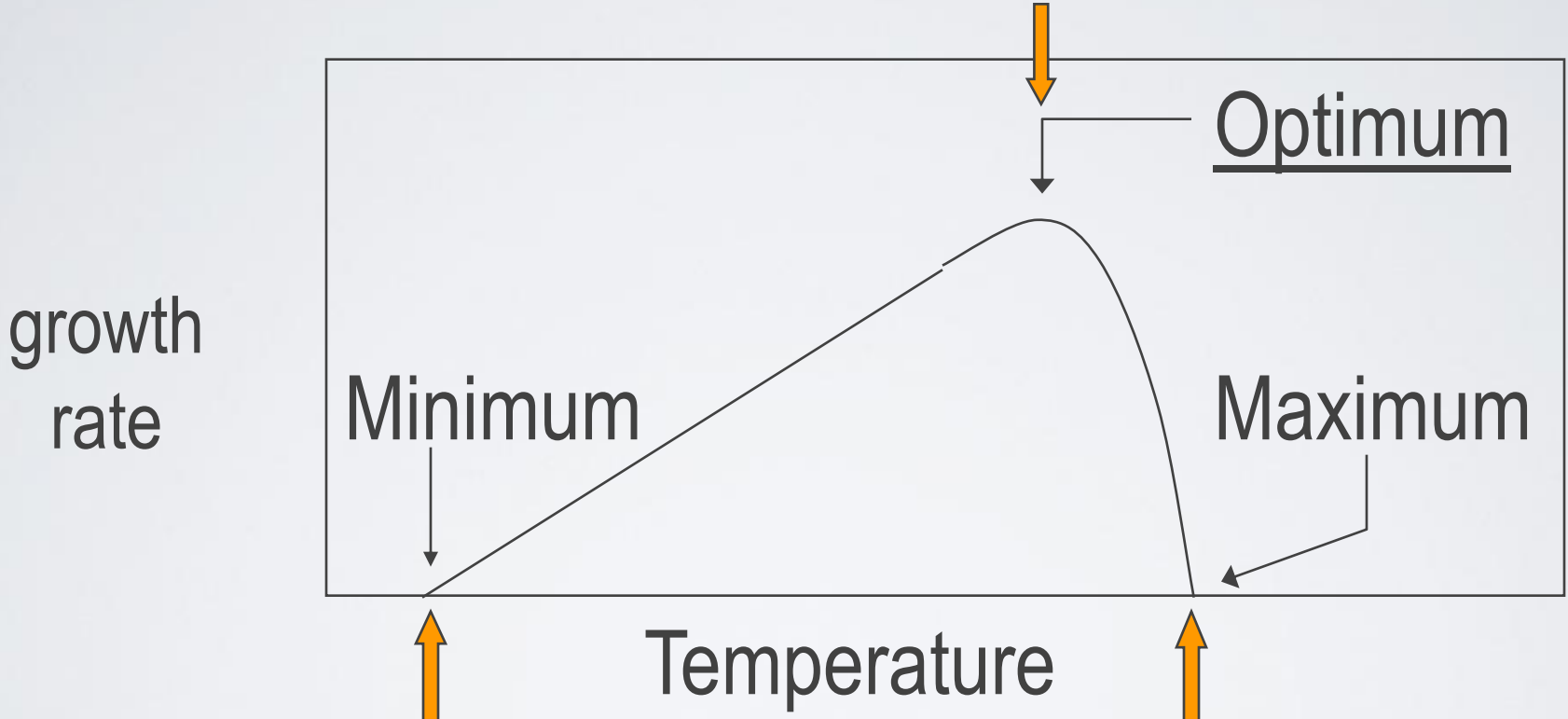
Bacteria Classification

- Thermophiles have growth minimal around 45°C, and optimal of 55 to 65°C
- Hyperthermophiles have growth minimal around 55°C and optimal of 80 to 110°C
- Stenothermal organisms have a narrow range of cardinal growth temperatures; eurythermal organisms have a wide range of cardinal growth temperatures



A. Cardinal temperatures for growth

At optimum temperature,
fastest growth rate



membrane "gels" and
does not function properly

proteins denature;
membranes break down

B. Classes of microbes based on optimal temperature

psychrophile mesophile thermophile hyperthermophile
<15 °C 20 - 50°C 50 - 80°C 80 - 113°C

