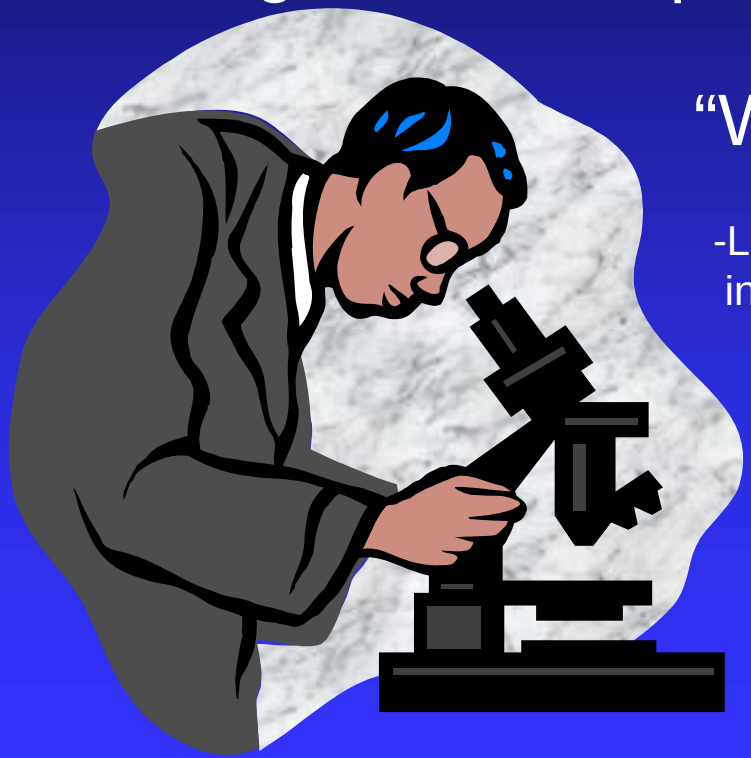
Basic Microbiology for Fresh Cut Produce

- Intro to the Microbial World
- Good 'Bugs' and Bad 'Bugs'
- Microbial Growth
- Factors that Influence Growth

MICROBIOLOGY

The study of small living organisms seen only by using a microscope.



"Wee Beasties"

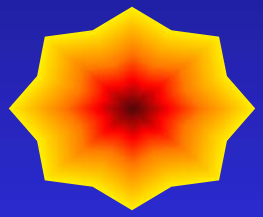
-Leeuwenhoek
inventor of the microscope

SEM images ©Dennis Kunkel, www.pbrc.hawaii.edu/kunkel/gallery

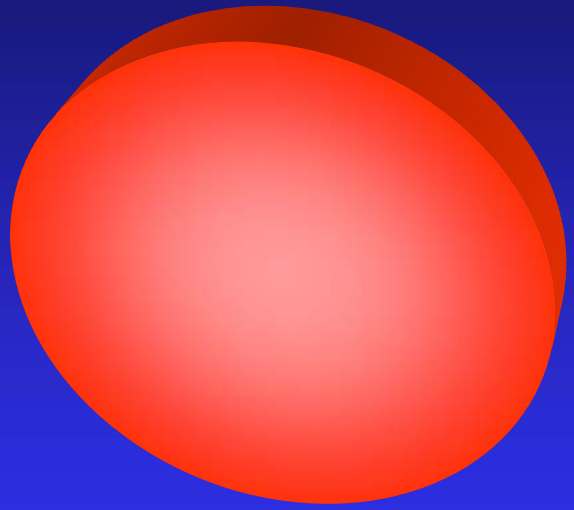
How Big Is A Cell?

Virus
1/10 micron

Salmonella
1.1 micron



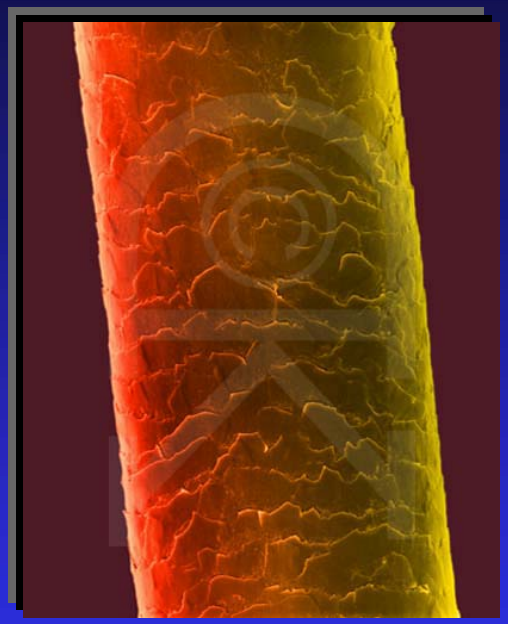
Toxoplasma Cyst
3.5 micron

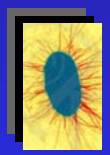


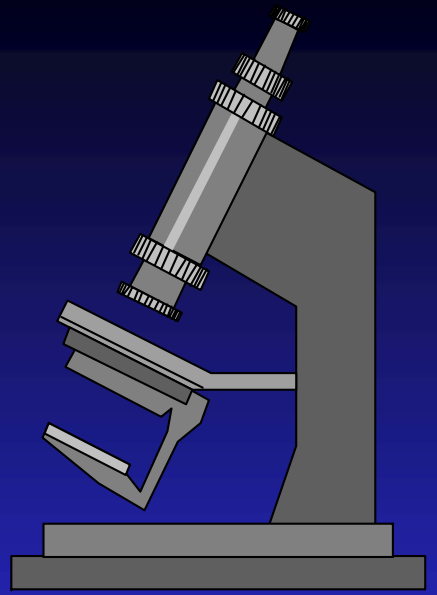
Human Red Blood Cell
9 micron

Average Human Cell
25-30 micron

Under a Microscope







Human hair
100 microns

Yeast
25 microns

Bacteria
0.5 micron

Virus
0.027
micron

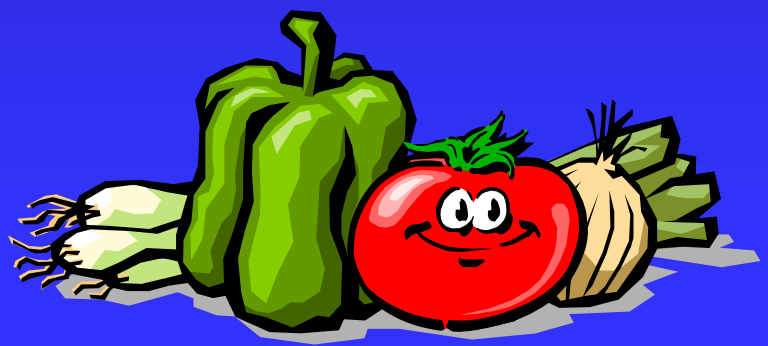
1 micron = 1/25,400 inch

Raw foods contain microorganisms

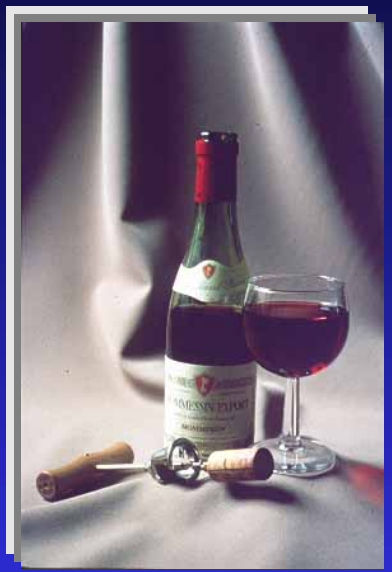
Microbes present an "invisible challenge”

⇨You can't see them

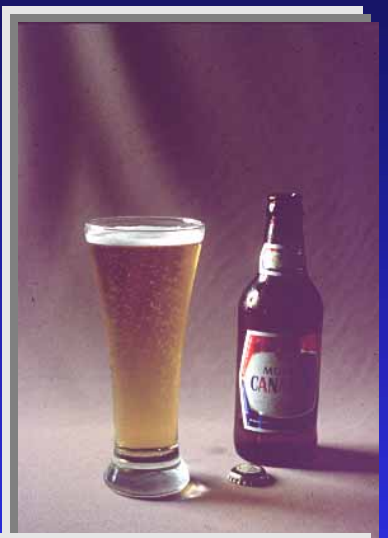
⇨They don't usually change the appearance, taste or odor of food.

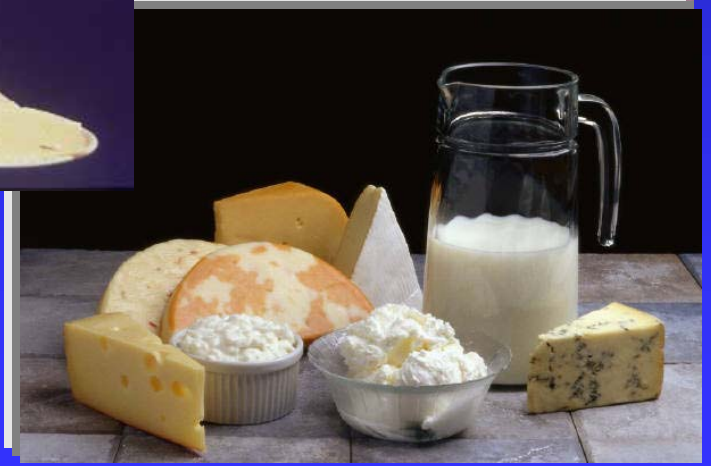


Good Things Microorganisms Do



Fermented Foods

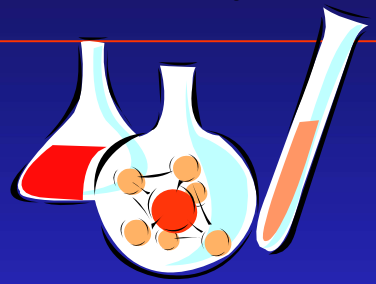




Good Things Microorganisms Do

Produce useful chemicals

antibiotics



enzymes

vitamins

Break down organic matter

Compost garbage, manure

Bad Things Microorganisms Do

Spoilage

- Reduced quality
- Shortened shelf-life
- Loss of revenue

Rots and Decay

Spoilage

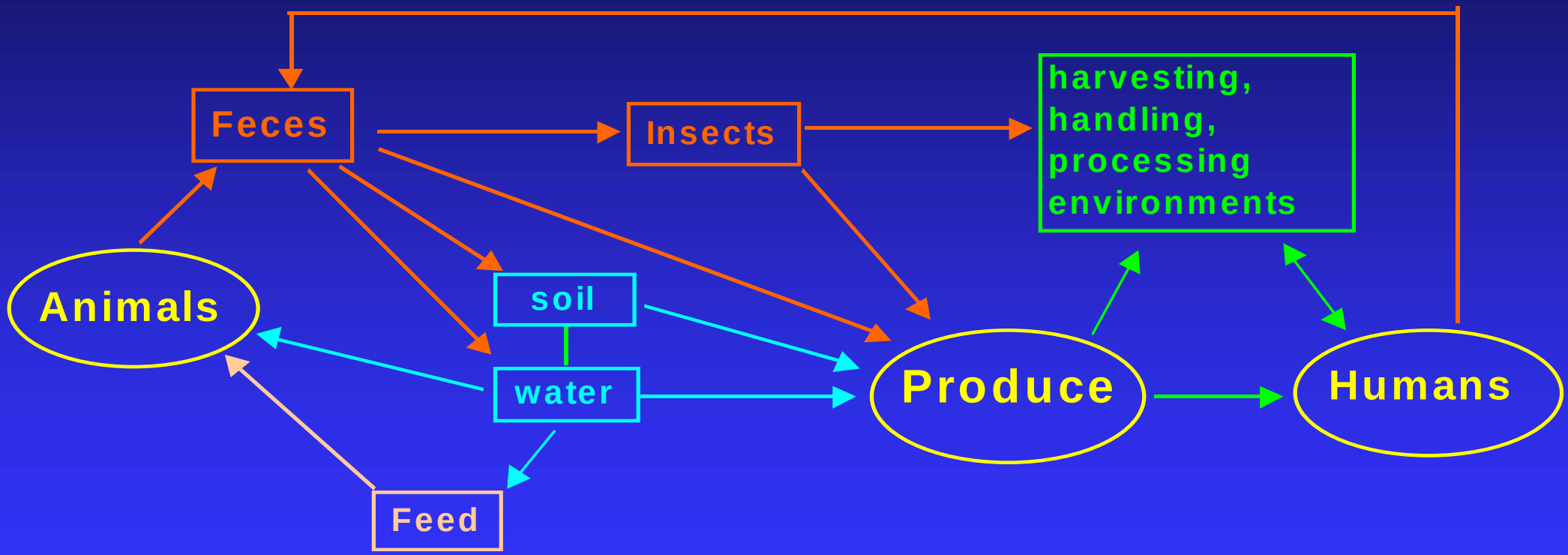
Spoilage Organisms Commonly Encountered in Fresh Cut Products

- **Common between 34 and 50°F (1.1 to 10°C):**
  - ***Pseudomonas* spp., *Erwinia herbicola*, *Flavobacterium*, *Enterobacter agglomerans*,**
  - **Lactic acid bacteria**
  - **True fungi and yeasts**

Microorganisms Can Cause Illness

- Few harmful to humans
- Few transmitted through foods

Sources of Contamination

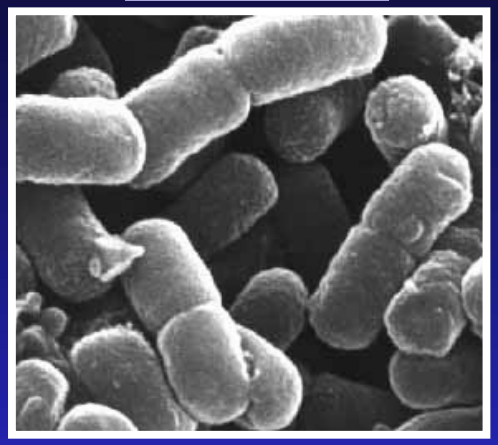


Adapted from Beuchat, 1996

Classification of Microorganisms

- Studied by
  - growth characteristics
  - visible features
  - DNA
- Named with
  - a genus and species designation
  - *Clostridium botulinum* (Botulism bacteria)
  - *Lycopersicon esculentum* (tomato)

bacteria



yeasts

molds

- Fresh Cut Quality – Bacteria, yeast, molds
- Fresh Cut Safety – Bacteria, viruses, protozoa

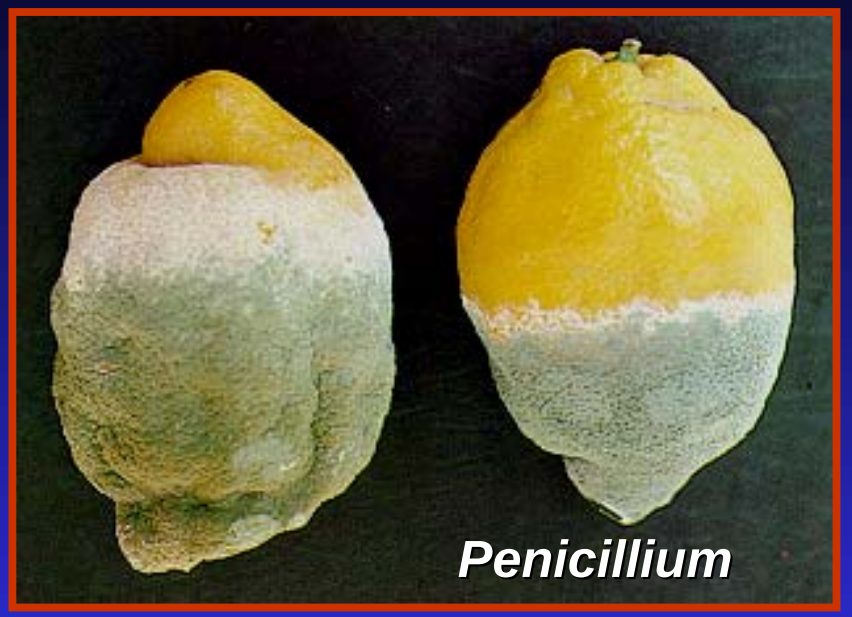
SEM images ©Dennis Kunkel, www.pbrc.hawaii.edu/kunkel/gallery

Molds

- Widely distributed in nature
- Grow on many substances
- More tolerant to cold than heat
- Most have low heat resistance
- Some have high chlorine tolerance

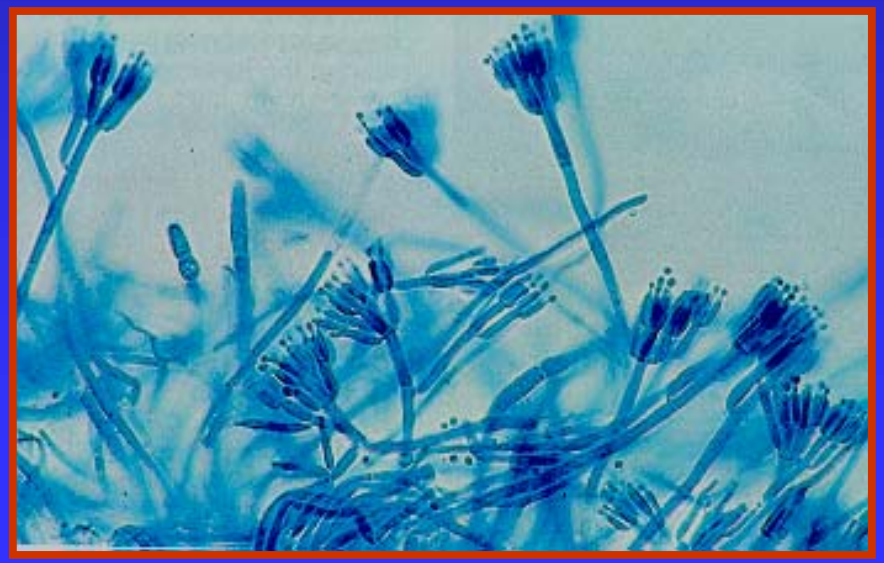
Images from http://www.fright.com/spam/homegrown3.html

True fungi



Primarily enter

- **direct penetration**
- **natural openings**
- **wounds**
- **quiescent infections**



Yeasts

- Like foods with sugar and acid

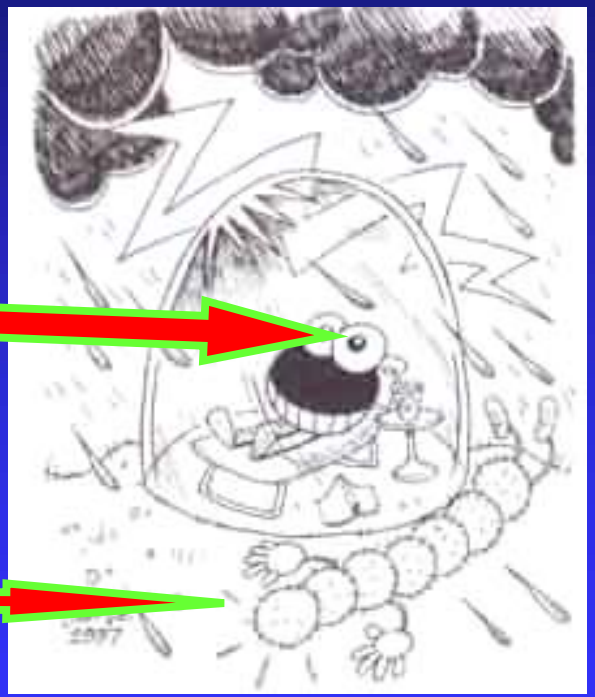
- More important on fruits
- Produce alcohol and carbon dioxide

Bacteria

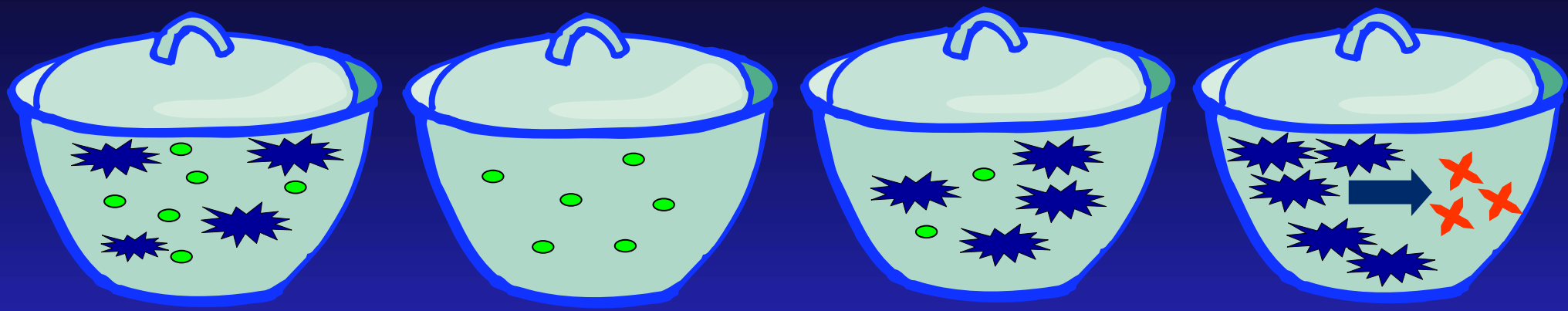
- Most important for vegetables
- Single-celled, may form chains
- Many shapes and forms
- Can produce spoilage enzymes
- Some produce toxins

Some Bacteria Form Very Resistant "Spores"

- Spore = dormant state
- Vegetative cell = active state
- Bacterial spores survive heat, cold, chemical agents
- Vegetative cells much more sensitive



Spore-forming Bacteria



Food with vegetative cells and spore cells

Heated to 165°F (74°C)

Spores Survive

Cooled

Spores become vegetative

Vegetative cells grow and produce toxin

Spore

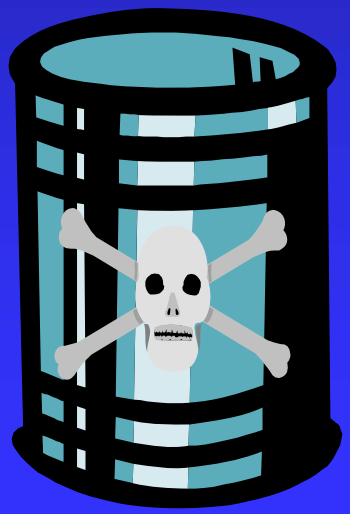
Vegetative cell

Growth

Toxins

Most Important Spore-forming Bacteria in Thermal Processing and Anaerobic MAP

Clostridium botulinum



a.k.a.

C. botulinum

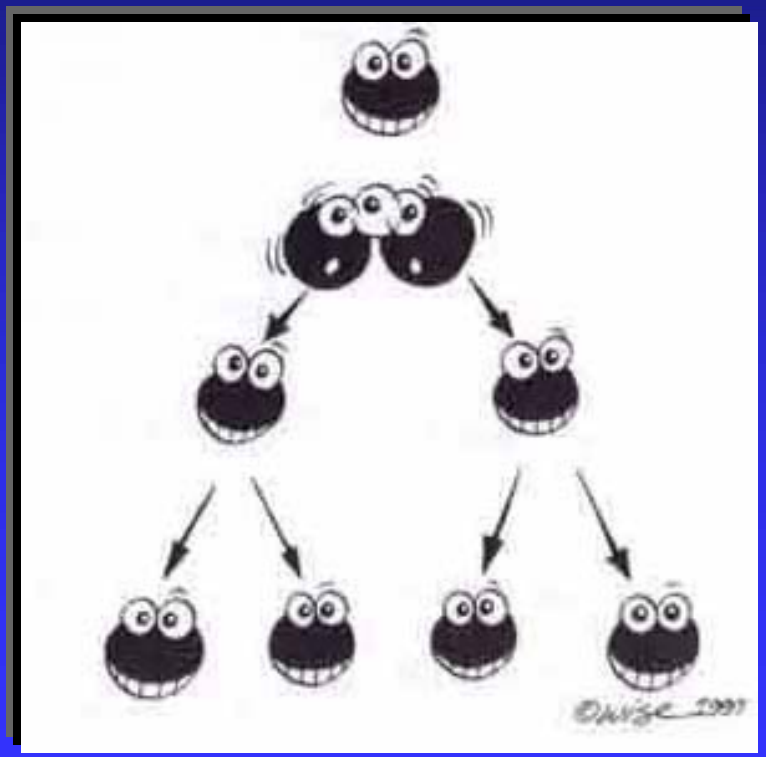
C. bot

Bot

Botulinum

Growth of Bacterial Cells

- Called “growth” or “multiplication”
- Under *the best* conditions a cell can divide every 20 to 30 minutes



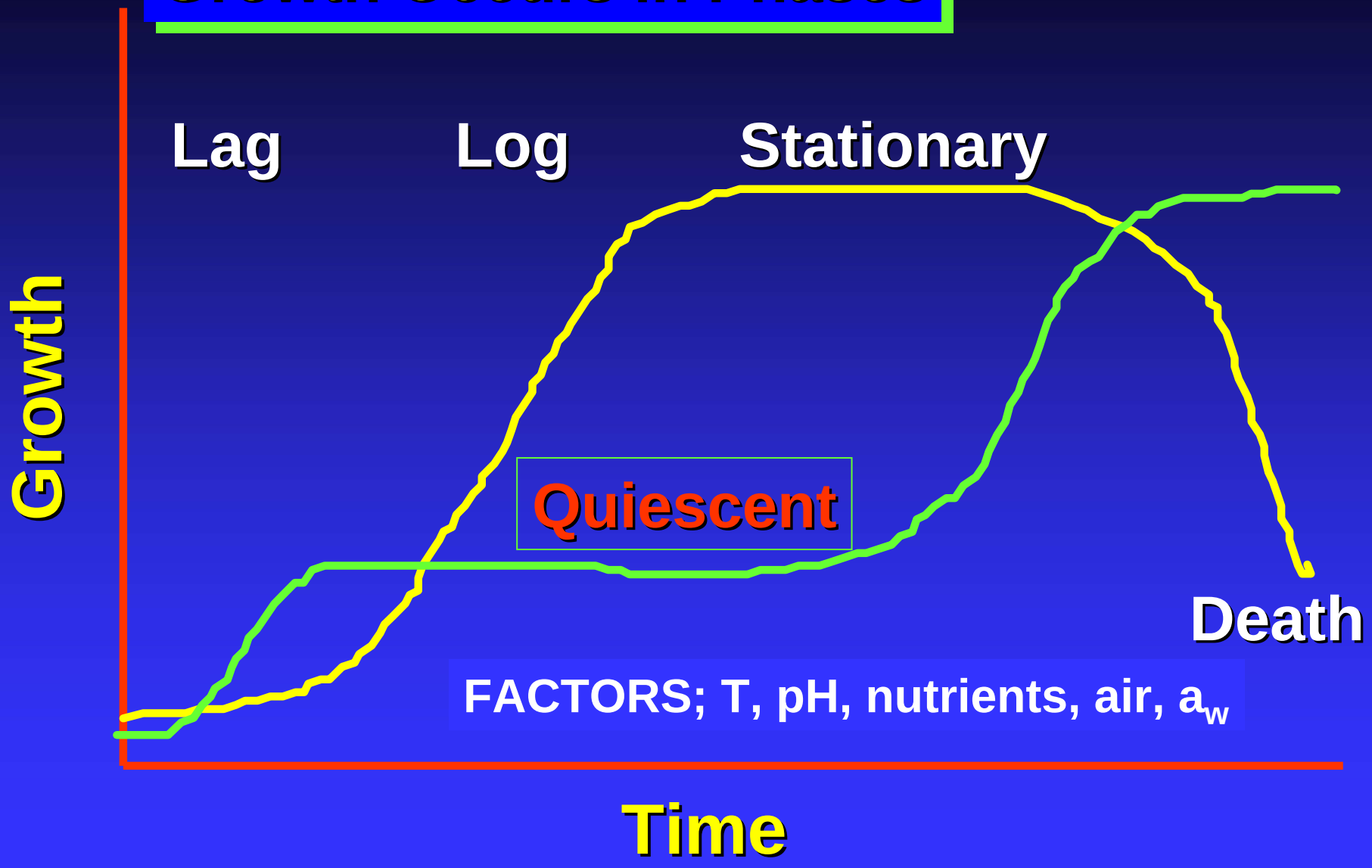
Reproduce by simple division

Multiplying Bacteria: 30 minute doubling time

- 8 a.m. 1 cell
- 9 a.m. 4 cells
- 10 a.m. 16 cells
- 11 a.m. 64 cells
- 12 p.m. 256 cells
- 1 p.m. 1024 cells
- 2 p.m. 4096 cells
- 3 p.m. 16,384 cells
- 4 p.m. 65,536 cells
- 5 p.m. 262,144 cells
- 6 p.m. 1,048,576 cells

So Why Don't Bacteria Take Over the World???

Growth Occurs in Phases



SPEED of Growth is Influenced by:

- ✓ Properties of the Produce

  Nutrients Moisture Acidity

- ✓ Properties of the Environment

  Temperature Relative Humidity Air

Growth Factors - Nutrition

- Different bacteria tend to have preferred “foods”
  - Presence of natural inhibitors
    - May slow or stop growth

- *Salmonella* is very versatile
  - Grows fast on simple sugars
  - Concentration dependent
    - Slow on lettuce
    - FAST on cantaloupe

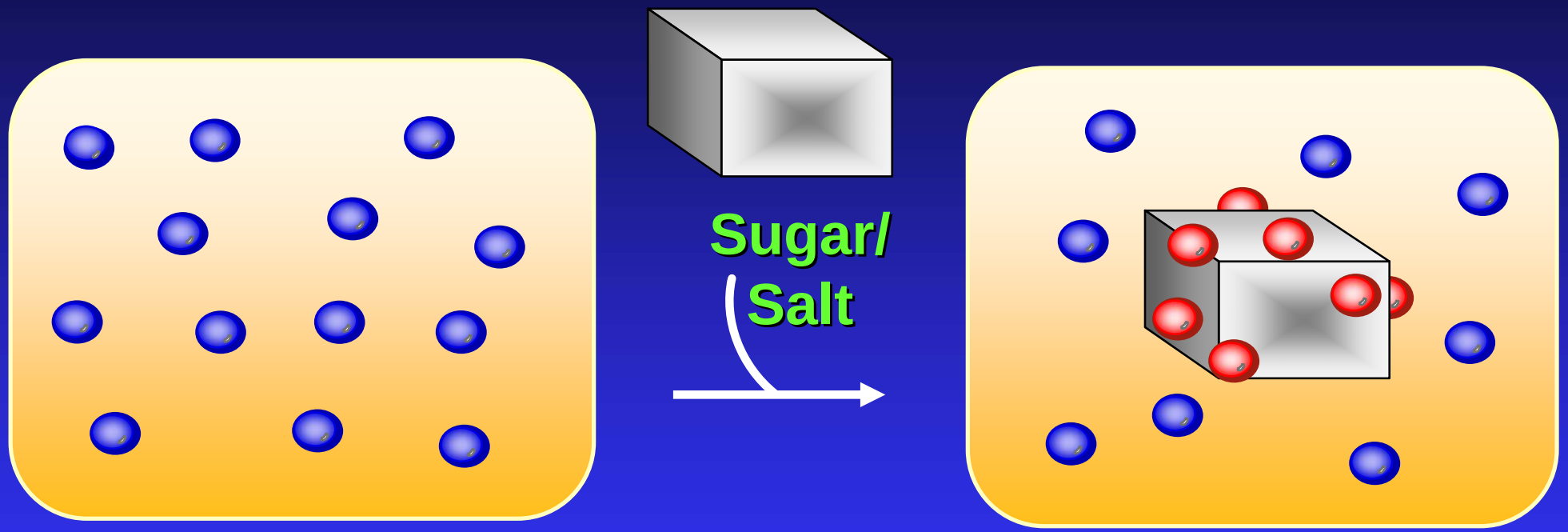
Bacteria Found "Internally" in Sound Vegetables

- *Citrobacter*
- *Coryneforms*
- *Enterobacter*
- *Erwinia*
- *Flavobacterium*
- *Lactobacillus*
- *Klebsiella*
- *Pantoea*
- *Proteus*
- *Pseudomonas*
- *Ranella*
- *Serratia*
- *Xanthomonas*

Growth Factors:Moisture

- Amount and availability of moisture
  - Most bacteria require free moisture
- Water activity
  - a measurement of available moisture in a food

Water Activity (a_w)



15 molecules total

15 molecules available

15 molecules total

8 molecules available

A Measure of Available Water

Water Activity of Some Foods

Food	a_w
Chopped Lettuce	0.98
Cheese Spread	0.94
Soy Sauce	0.80
Fudge Sauce	0.83
Soft Moist Pet Food	0.83
Peanut Butter	
15% Total Moisture	0.70
Dry Milk 8% Total Moisture	0.70
Livewurst	0.96
Salami	0.82

Not a significant factor in fresh cut produce

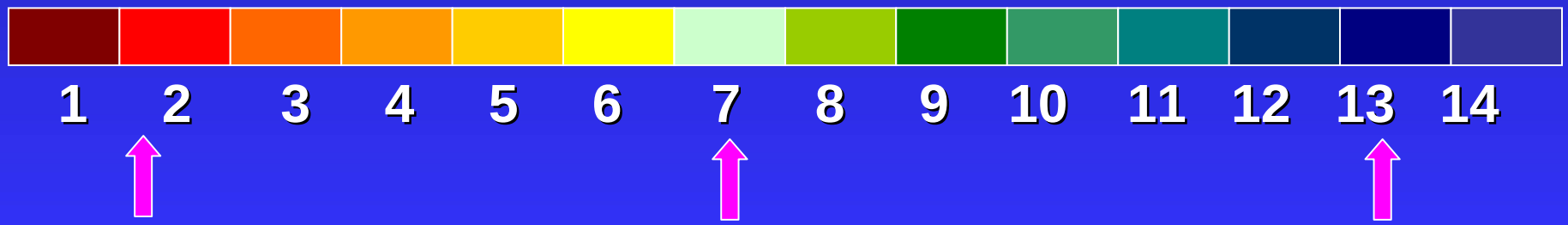
Most cut vegetables 0.99
cut fruit 0.97 to 0.98

Growth Factors: pH

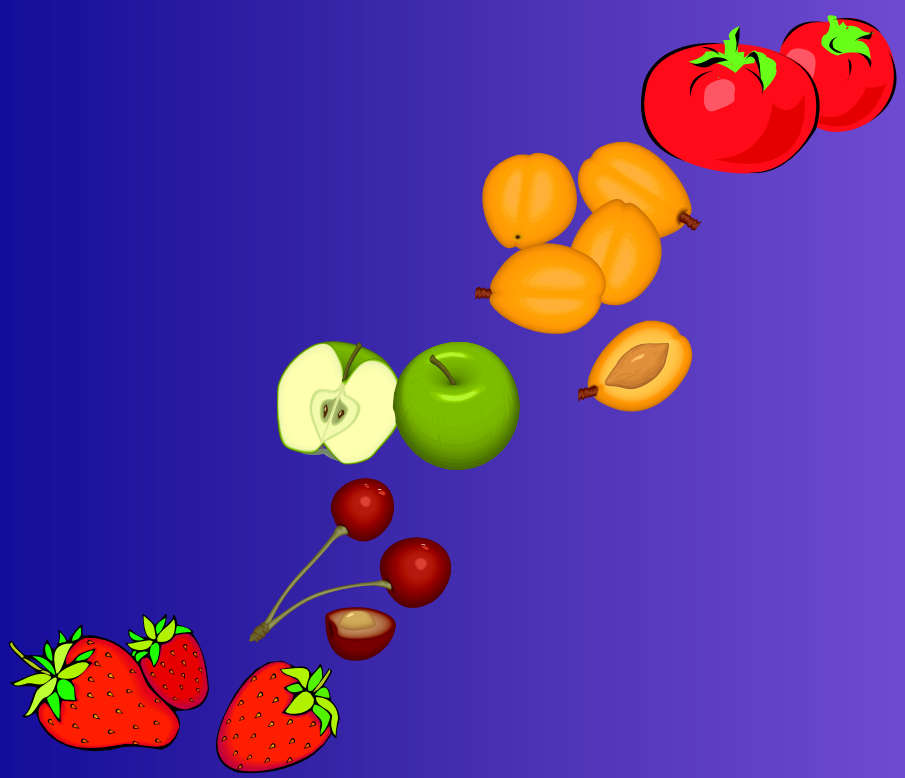
pH is a measure of acidity

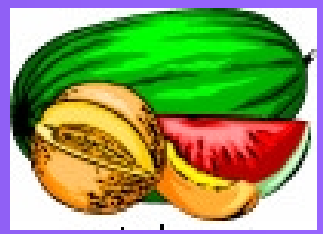
pH = -log (H^+ ions)

Scale ranges from 0 to 14



pH and Fresh Cut Raw Materials





pH 3 pH 4.6 **pH 7**

High acid food *Low acid food*

Limits of pH for Growth of Some Key Microorganisms

Microbe	pH range
Candida spp. (yeast)	2.3-8.8
Clostridium botulinum	4.7-8.5
Escherichia coli	4.4-9.0
Escherichia coli O157:H7	4.2-9.0
Erwinia carotovora	5.6-9.3
Lactobacillus spp.	3.8-7.2
Pseudomonas marginalis	6.0-8.5
Salmonella spp.	4.8-8.0

Limits of pH for Growth of Key Fungi

Microbe	pH range
Alternaria alternata	2.7 - 8.8
Aspergillus oryzae	1.6 - 9.3
Botrytis cinerea	3.5 - 8.8
Candida spp.	2.3 - 8.8
Fusarium oxysporum	1.8 - 11.1
Penicillium italicum	1.9 - 9.3
Sclerotinia sclerotiorum	2.2 - 9.3

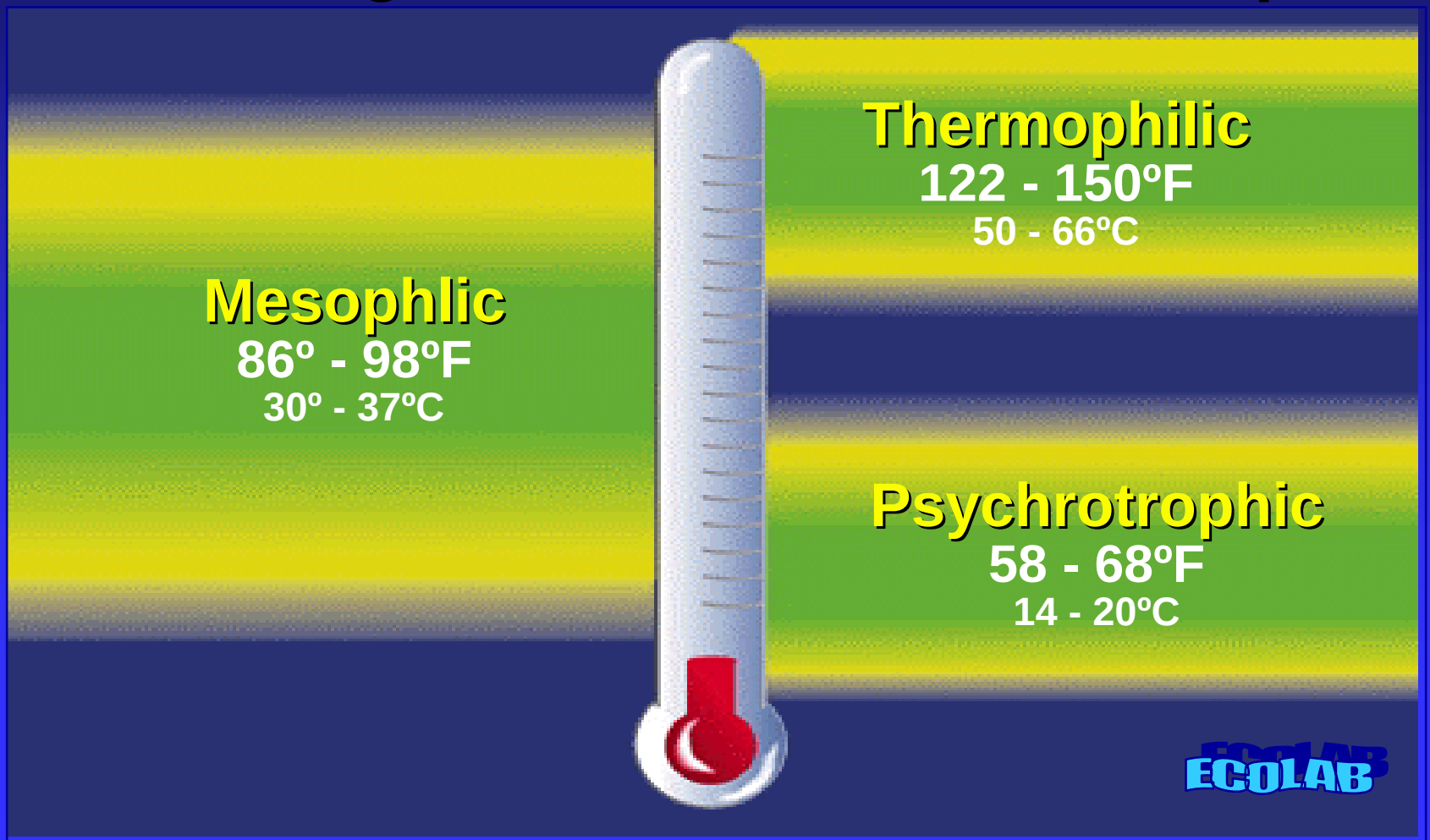
Effect of Mold Growth

- Molds can consume acid
  - may raise food pH to above 4.6

- *C. botulinum* may grow in moldy acidic foods

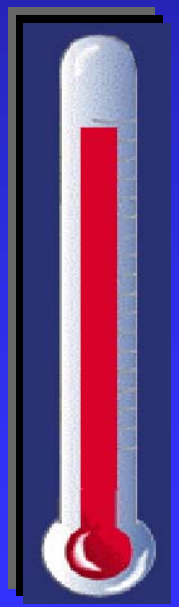
Growth Factors – Temperature

Microorganisms are in Three Groups

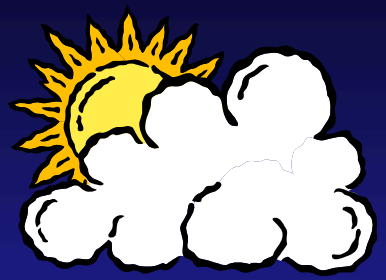


The Thermophilic Group

- Grow best at 122 ºF to 155 ºF (50 – 68C)
- Spores are very heat resistant
  - May survive processing
  - Can’t grow at normal storage temperatures
- No disease-causing organisms
- Not a concern for fresh cut

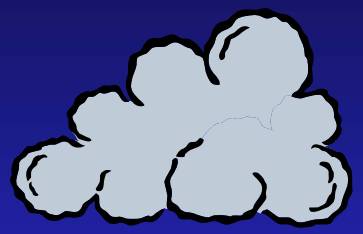


The Mesophilic Group



- Grow best between 86 ºF to 99 ºF (30-37C)
- All microorganisms that cause illness
- Many cause spoilage
- Typical of Aerobic Plate Count tests

The Psychrotrophic Group



- Grow best between 58º to 68ºF (14-20°C)
- Can grow slowly at 40ºF (4.4°C)

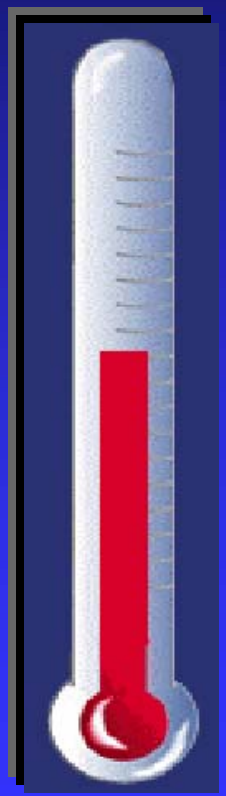
- Some capable of growth at 30°F (-1°C)
  - Problems in garlic storage
- Spoilage – *Pseudomonas fluorescens*
- Decay Pathogen – *Botrytis cinerea*
- Human Pathogen – *Listeria monocytogenes*

Psychrotrophic pathogens

- **Microorganisms that can grow at refrigeration temperatures are of concern, because they may multiply to high levels during extended product shelf-life**
  - ***Listeria monocytogenes,***
    non-proteolytic *Clostridium botulinum* (some types),
    ***Yersinia entercolitica*,**
    Aeromonas hydrophila

Temperature and Microbial Risk

- "Danger zone" 41 to 140°F (5 to 60°C)
  - Most problematic microbes



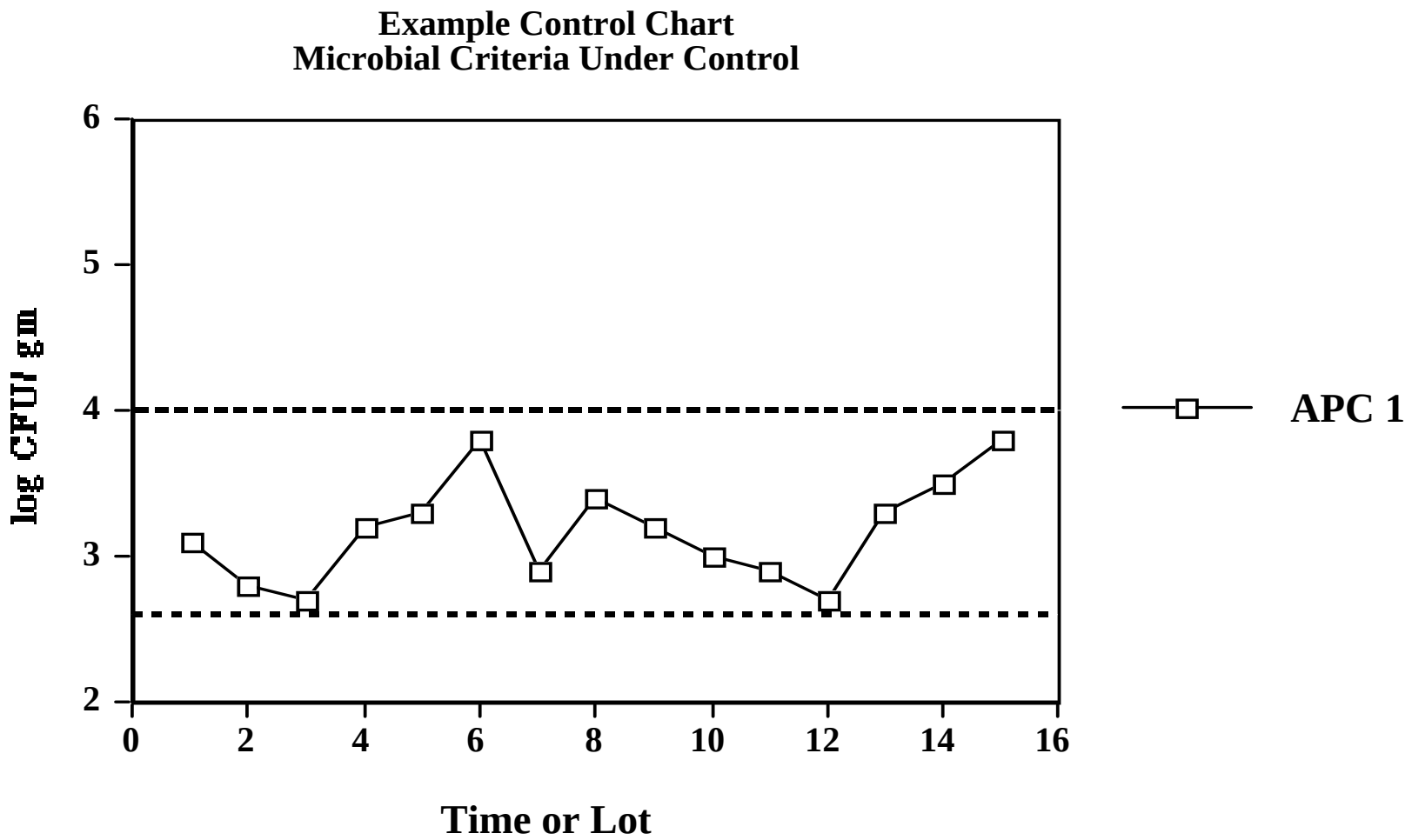
Growth Factors - Air

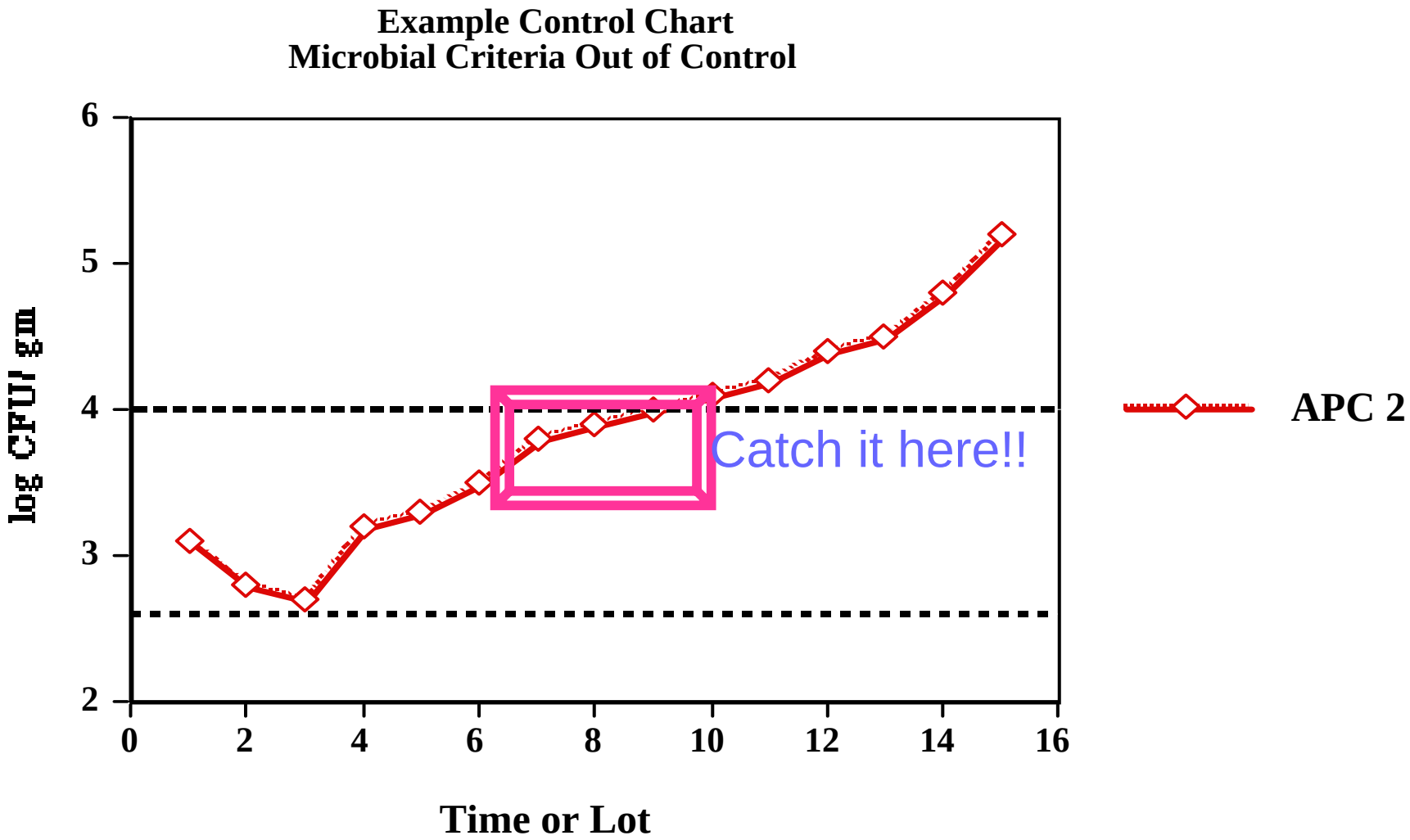
- **Aerobes**
  Oxygen necessary
  - *Pseudomonas*
- **Anaerobes**
  Oxygen toxic
  C. botulinum
- **Facultative**
  Either way is OK
  - *Erwinia, E. coli, Salmonella*

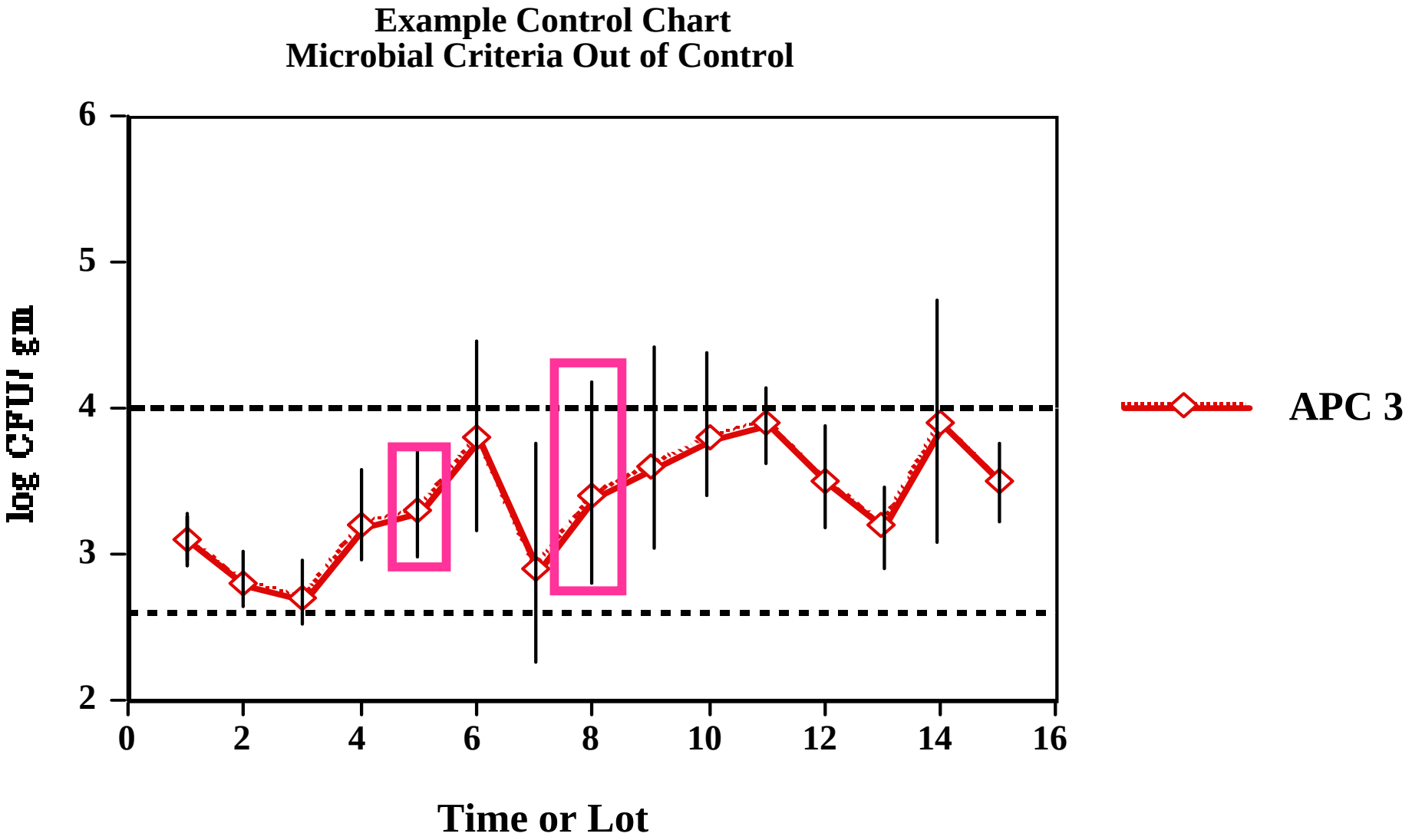
Microbial Pathogens - MAP

- Some foodborne pathogens can multiply in MAP conditions.
- Many spoilage organisms are inhibited.
  - The product would appear acceptable, even when contaminated with a high level of pathogen

General Microbe Testing: Counts, Cleanliness, Pathogen Detection




Example Control Chart
Microbial Criteria Out of Control
log CFU/ gm
6
5
4
3
2
0
2
4
6
8
10
12
14
16
Time or Lot
Catch it here!!
APC 2


Example Control Chart
Microbial Criteria Out of Control
log CFU/ gm
6
5
4
3
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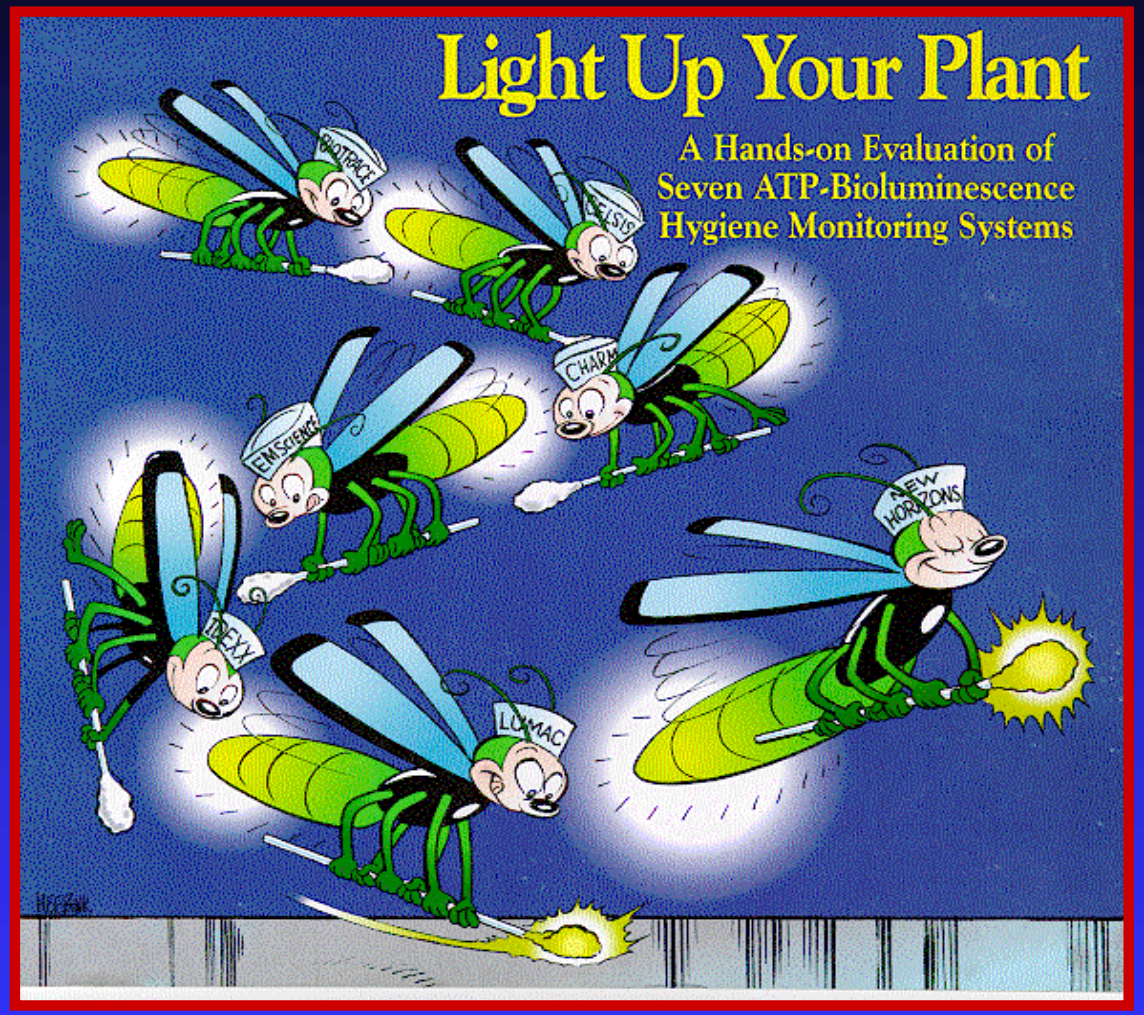
4
6
8
10
12
14
16
Time or Lot
APC 3


Light Up Your Plant
A Hands-on Evaluation of
Seven ATP-Bioluminescence
Hygiene Monitoring Systems
BIOTRACE
CHARM
EMSCIENCE
NEW HORIZONS
IDEXX
LUMAC


LIGHTNING

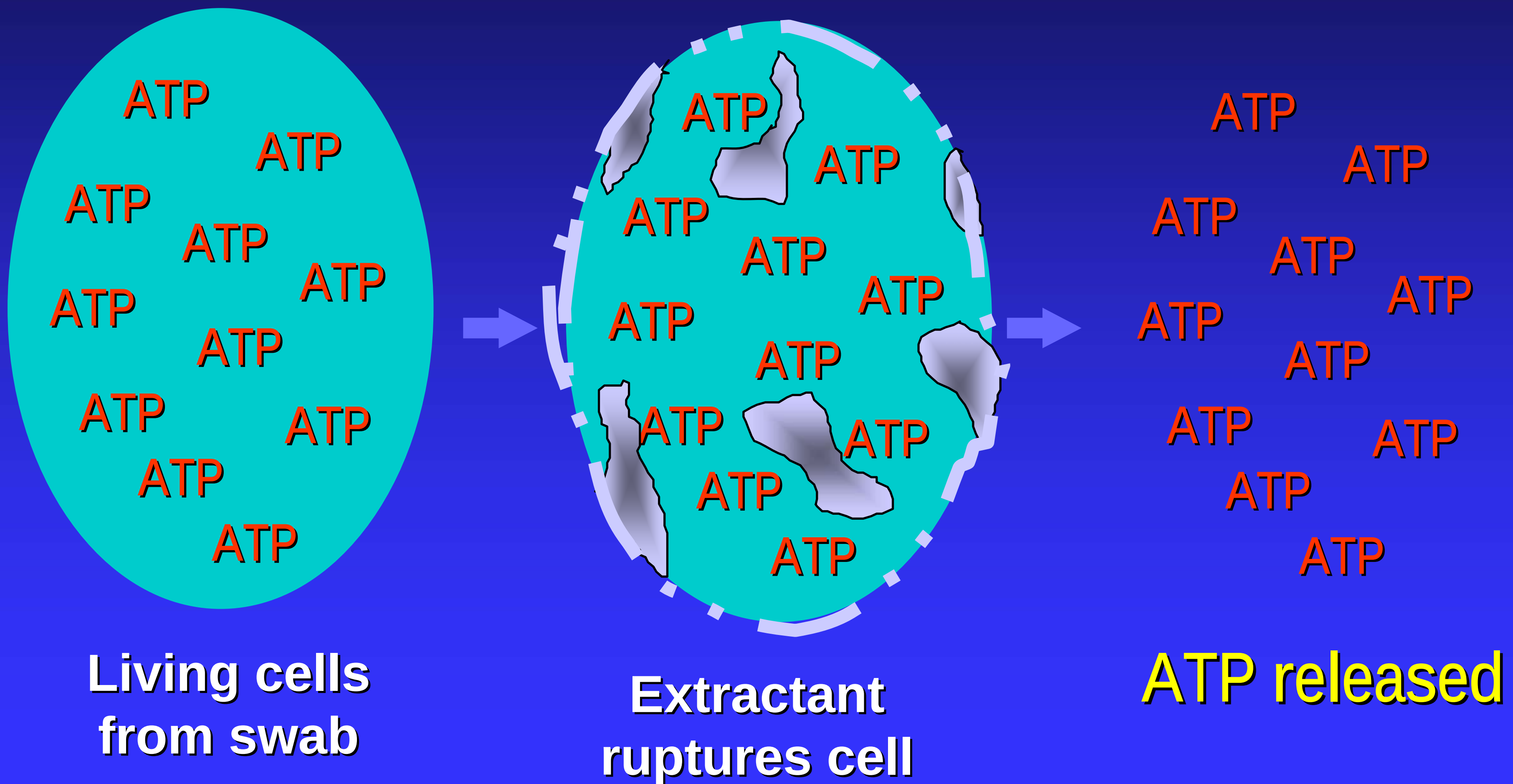
Principles of ATP Bioluminescence

- Adenosine triphosphate (ATP): universal energy donor
- Living cells have ATP in relatively consistent amounts
- Enzymes in firefly converts chemical energy (ATP) to light
- Amount of light is directly proportional to amount of ATP present
- Not specific for microbial ATP

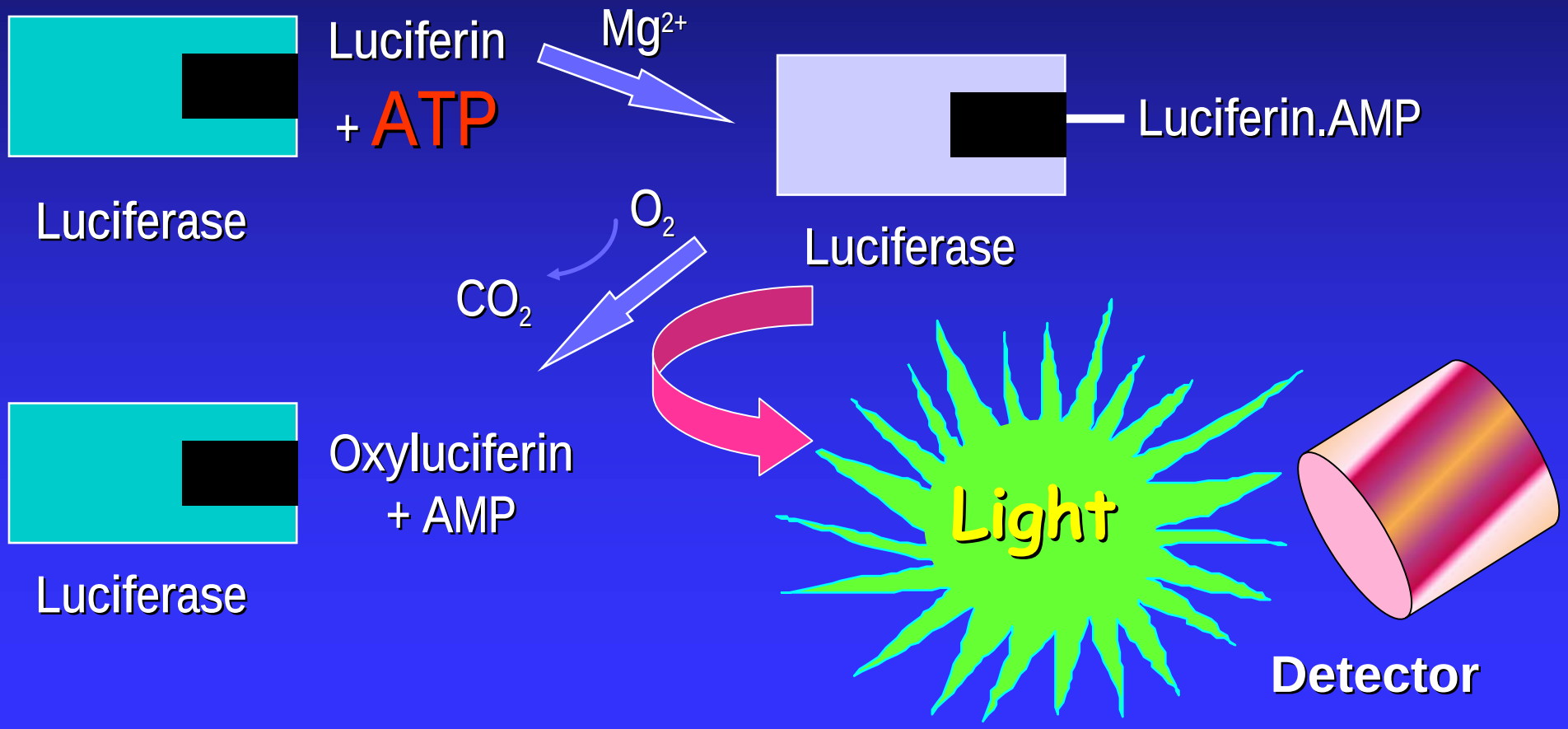
The ATP Bioluminescence Reaction

- 1 Photon of Light = 1 Molecule of ATP
- Light output is measured in Relative Light Units (RLU)
- Results are rapid, usually within minutes
- Pass / No Pass

ATP Bioluminescence



The ATP Bioluminescence Reaction



Pathogen Detection

- Pathogens typically
  - low level, sporadic

- Specific for amount tested
- Positive or negative (not numbers)
- May take several days for test
- Positive requires immediate action
- Negative says little about lot

Summary

- Microorganisms are found everywhere
- Some microorganisms are beneficial, others cause spoilage or human illness
- Microorganisms need appropriate moisture, nutrients, pH, air, temperature and TIME