UNIT 3
METABOLISM & GROWTH REQUIREMENTS

Properties of enzymes: Control Metabolic Pathways

- Enzymes (type of protein)
  - Act as catalysts (they speed up chemical reactions up to a million times without changing)
  - Activation energy is needed to get a reaction started
  - One way to activate a reaction: heat (not good)
  - Function #1: enzymes help lower the activation energy so that reactions happen at mild temperatures in cells

How enzymes work

- www.youtube.com/watch?v=tI69AVRW0DU

Properties of enzymes

- High degree of specificity
  - Catalyze only one type of reaction
  - Work on only one particular substrate

- Recognized by the suffix -ase
  - Lipases breakdown lipids
  - Peptidases break peptide bonds

- Many need the presence of a cofactor or coenzyme:
  - Coenzyme: a nonprotein molecule associated with the enzyme (made by vitamins…yep, they’re important)
  - Cofactor: an inorganic molecule that improves the fit of the enzyme & substrate (like Mg+)
Properties of enzymes

Factors that affect enzymatic reactions
- pH
- Temperature
- Concentration of substrate, product, and enzyme

Enzyme Inhibition:
- Competitive inhibitor: molecule similar to the substrate structure binds to the active site to prevent the binding of the substrate; reversible
- Noncompetitive inhibitor: bind to an allosteric site (a site other than the active site) and changes the shape of the active site; irreversible or reversible
- Feedback inhibition: when the amount of product influences the enzyme that started the process to stop

Brain Check

1. Which of the following would influence the rate of an enzyme reaction?
   a. Temperature
   b. pH
   c. Concentration of substrate molecules
   d. Concentration of product molecules
   e. All of these

2. The major energy-exchange molecule in living cells is

Microbial Metabolism

- Microorganisms have to eat too...
- Autotrophs: self-feeders that use CO₂ to make organic molecules/food
- Photoautotrophs: use sunlight (don’t usually cause disease)
- Chemoautotrophs: use inorganic substances/chemicals like sulfides & nitrates

Microbial Metabolism

- Heterotrophs: other-feeders that use already made organic molecules/food
- Photoheterotrophs: use sunlight for nutrition, but have to supplement with organic molecules
- Chemoheterotrophs: use organic compounds like proteins, fats, & carbohydrates

Microbial metabolism & energy

- Microorganisms have to capture energy for several things (movement, body functions, etc)
- Cellular respiration: process of organisms breaking down food into ATP
- Purpose of ATP: energy is stored in this molecule

The Need for Energy

ATP

Adenosine

Phosphate

Phosphate

Phosphate

High-energy bond: Stores a lot of energy that is released when the bond is broken

ADP

Adenosine

Phosphate

Phosphate

Energy
Three Stages of Cellular Respiration in microbes

<table>
<thead>
<tr>
<th>Stage</th>
<th>Aerobic/Aerobic</th>
<th>Oxygen required?</th>
<th>Location</th>
<th>ATP Produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Glycolysis</td>
<td>Anaerobic</td>
<td>No</td>
<td>Cytoplasm</td>
<td>2</td>
</tr>
<tr>
<td>2. Krebs Cycle</td>
<td>Aerobic</td>
<td>Yes</td>
<td>Cytoplasm</td>
<td>2</td>
</tr>
<tr>
<td>3. Electron Transport</td>
<td>Aerobic</td>
<td>Yes</td>
<td>Cell Membrane</td>
<td>34</td>
</tr>
</tbody>
</table>

FERMENTATION
- Occurs after glycolysis if oxygen remains absent
- Doesn’t yield a lot of energy
- Glycolysis (2 ATP) + Fermentation (2 ATP) = 4 ATP

<table>
<thead>
<tr>
<th>Alcoholic fermentation</th>
<th>Lactic Acid fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvic Acid → ethyl alcohol + carbon dioxide</td>
<td>Pyruvic Acid → lactic acid</td>
</tr>
</tbody>
</table>

- Carried out by yeast and some bacteria
- Used in brewing beer, making wine, and baking bread and cakes
- Occurs in lactobacilli, streptococci, and mammalian cells
- Used to make cheese (lactobacilli) and that burn you feel after working out

Other types of fermentation

Fermentation Taken Too Far?
- Casu Marzu: Forbidden in the U.S.

Reason: Casu marzu, a traditional Sardinian cheese, develops when cheese fly larvae are introduced into Pecorino to promote advanced fermentation. As the larvae hatch and eat through the cheese, it softens. Diners have to dig in before the maggots die. Casu marzu, like many unpasteurized cheeses, is banned in the U.S.

Cellular Respiration summarized

**Anaerobic: no oxygen present**

- 1 molecule of GLUCOSE
- Glycolysis: Anaerobic Makes 2 ATP
- Lactic Acid: produces lactate
- Alcoholic: produces alcohol & CO₂

Anaerobic Respiration = 4 ATP

**Aerobic: oxygen is present**

- Oxygen available
- Krebs Cycle: Aerobic Makes 2 ATP
- Electron Transport Chain: Aerobic Makes 34 ATP

Aerobic Respiration = 38 ATP

Brain Check

1. The major energy-exchange molecule in living cells is
   a. ADP
   b. AMP
   c. ATP
   d. Glucose

2. Why does fermentation occur?
   a. Because oxygen is absent
   b. Because oxygen is present
GROWTH OF BACTERIA

Microbial cell division
- Binary fission: [www.youtube.com/watch?v=yToii3.p-Ni](www.youtube.com/watch?v=yToii3.p-Ni)
- Budding: [vimeo.com/39669636](vimeo.com/39669636)

Growth and Cell Division
- **Microbial growth**: the increase in the number of cells as they divide (not their actual size)
- **Binary Fission**: when a cell duplicates or copies itself and then divides into 2 cells (how bacterial cells divide)
- **Budding**: when a small, new cell develops from the surface of an existing cell and then separates from it (how yeast & a few bacterial cells divide)

Bacterial growth curve
- To help bacterial cells grow, you put them into a fresh, nutrient-rich mixture of substances on or in which microorganisms grow. This is called **medium (media)**.
- The length of time required for one cell division is called the **generation time**.
  - For most bacteria, it’s between 20 min-20 hrs
- It happens at an **exponential or logarithmic** rate
  - Ex. A culture with 1000 organisms per mL with a generation of 20 min would contain 2000/mL after 20 min, 4000 after 40 min, and so on

Generation Times practice questions
1. If an *E. coli* culture has a doubling time of 20 minutes, how many cells will result after 1 hour from a single cell?
2. If an *S. thermophilus* culture has a doubling time of 30 minutes, how many cells will result after 3 hours from a single cell?
3. If an *L. acidophilus* culture has a doubling time of 25 minutes, how many cells will result...
Bacterial growth curve

- Cells in a nutrient-rich environment will display 4 general phases of growth:

1. **Lag Phase**: organisms are metabolically active—growing and synthesizing various substances, but not increasing in number

2. **Log Phase**: organisms divide at an exponential or logarithmic rate with a constant generation time

3. **Stationary Phase**: the # of new cells = the # of cells dying; the medium contains limited nutrients and may even have some toxic waste materials

**Brain check**

1. Budding is seen in _______ cells.
   a. Animal
   b. Bacteria
   c. Viruses
   d. Yeast

2. Binary fission is seen in _______ cells.
   a. Animal
   b. Bacteria
   c. Viruses

**Brain check**

3. When a cell divides, it is referred to as the _____.
   a. Daughter cell
   b. Dividing cell
   c. Father cell
   d. Mother cell

4. The newly created cells are known as _______.
   a. Daughter cells

**Measuring bacterial growth: Counting Large Populations**

- **Serial Dilutions/Standard plate counts**:
  1. Successive 1:10 dilutions of a liquid culture of bacteria are made and transferred onto an agar plate (start with 1:100 dilution)
  2. Each colony that grows represents one live cell from the original sample
Physical Influences on Growth

1. **pH**
2. **Temperature**
3. **Oxygen concentration**
4. **Moisture**
5. **Hydrostatic Pressure**
6. **Osmotic Pressure**
7. **Radiation**

**Physical Influences # 1: pH**
- Remember that acidity or alkalinity of a medium is expressed in terms of pH.
- Microorganisms have an **optimum pH** (when they grow best); this tends to be neutral (pH 7).

  - **Acidophiles**: acid-loving; can make acids; exist from pH 0.1-5.4
    - Ex. *Lactobacillus* species (makes lactic acid)
  - **Neutrophiles**: exist from pH 5.4-8
    - Ex. Most bacteria that cause human diseases
  - **Alkaliphiles**: alkali (or base) loving; exist from pH 7-11.5
    - Ex. *Vibrio cholerae*

**Physical Influences # 2: Temperature**
- Most microorganisms can grow over a 30°C range.

  - **Psychrophiles**: cold-loving; 15°C-20°C (even 0°C)
    - *Obligates*: can’t grow above 20°C
    - *Facultative*: grow best below 20°C, but can above that
    - Habitat: soil & cold water (sea water can be below 0°C)
  - **Mesophiles**: moderate-loving; 25°C-40°C
    - Habitat: humans (our 37°C body temp is ideal)
  - **Thermophiles**: heat-loving; 50°C-60°C (even 110°C)
    - *Obligates*: have to grow above 37°C
    - *Facultative*: grow above or below 37°C
    - Habitat: compost heaps & hot springs

**Physical Influences # 3: Oxygen**

1. **Aerobes**
   - Use O₂ in their metabolic pathways

2. **Obligate Aerobes**
   - Need free O₂
   - Ex. *Pseudomonas* (common nosocomial)

3. **Microaerophiles**
   - Grow best in a small amount of free O₂
   - Ex. *Campylobacter* (causes intestinal)

4. **Anaerobes**
   - Do not use O₂ in their metabolic pathways
   - Ex. *Clostridium tetani* (lockjaw)

5. **Facultative Anaerobes**
   - Can switch between aerobic metabolism if O₂ is present or anaerobic if it isn’t
   - Ex. *Staphylococcus* & *E. coli*

6. **Aerotolerant Anaerobes**
   - Metabolize anaerobically, but aren’t harmed by free O₂.
Other Physical Influences on Growth

- Moisture: all microbes need a water environment
- Hydrostatic pressure: exerted by standing water; some bacteria are the only things that can survive deep in the ocean
- Osmotic pressure: diffusion of water; halophiles love high salt contents that would cause our cells to crenate or shrink

Microbial growth

- There are a few microbes called fastidious microorganisms because it is hard to meet their needs in the laboratory setting such as N. gonorrhoeae which grow well in the body, but not on media.

Nutritional Factors Affecting Bacterial Growth

- C, N, S, P availability: energy source, synthesis of organic molecules & cell components
- Trace elements: ions that serve as cofactors in enzymatic reactions
- Vitamins: used as coenzymes in enzymatic reactions; most microbes make their own.
  - Microbes in our intestines make Vitamin K (blood clotting) and Vitamin B
- Nutritional complexity: determined by enzymes

Brain Check

1. What are some physical requirements that must be satisfied to successfully culture bacteria?
2. What are some nutritional requirements that must be satisfied to successfully culture bacteria?
**Culture Media**

- Bacteria have to be grown (cultured) for them to be identified.
- By appropriate procedures, they have to be grown separately (isolated) on culture media and obtained as pure for study (Koch’s postulates)
- **Stock cultures**: maintained for keeping specimens viable for subculture into fresh medium for routine work
- **Preserved cultures**: maintained for storage to prevent contamination or change of characteristics

**Applications of Culturing**

- Maintaining cultures
- Preserved cultures
- Diagnostic tests
- Limitations on Culture

**Types of Culture Media—Consistency**

- **Solid media** — contains 2% agar
  - Colony morphology & pigmentation
  - Eg: Nutrient agar, Blood agar
- **Liquid media** — no agar
  - For inoculum preparation, blood culture, for the isolation of pathogens from a mixture
  - Eg: Nutrient broth
- **Semi solid medium** — 0.5% agar
  - Eg: Motility medium

**Culturing — Broth technique**

**Culturing — Plate techniques**

- **Streak Plates**: spreading bacteria across sterile agar so that the progeny of a single cell can be picked up from the surface and transferred to the sterile medium
Culturing – Plate techniques

- **Pour Plate Method**: using serial dilutions and transferring a portion of the solution to melted agar and then picking cells from a colony on agar.

Brain Check

1. What is the difference between solid and liquid media? When should you use a solid media? A liquid media?

2. The pour plate method is used with serial dilutions to find out what?
   a. That bacteria are present
   b. The number of bacteria per dilution
   c. IDK...I’m tired...I can’t do this right now

**Types of Media—Diagnostic**

**Synthetic or defined media (most common)**
- Media prepared from pure chemical substances and its exact composition is known
- Ex: Nutrient Broth (NB), Nutrient Agar (NA)
  - NB consists of peptone, meat extract, NaCl,
  - NB + 2% agar = Nutrient agar

**Complex media**
- Media other than defined media
- They have added ingredients
- Provide special nutrients
- Selective, differential, and enrichment media are examples

**Selective media**
- Encourages the growth of some organisms and inhibits others

Examples
- Mac Conkey’s medium for gram negative bacteria
- TCBS – for V.cholerae
- LJ medium – M.tuberculosis
- Wilson and Blair medium – S.typhi
- Potassium tellurite medium – Diphtheria bacilli

Mac Conkey’s medium
TCBS Thiosulfate Citrate Bile Salts Sucrose Agar
Potassium Tellurite media
LJ media
**Differential media**
- A media which has substances incorporated in it enabling it to distinguish between bacteria.
- Eg: CHROMagar

**Types of Media—Diagnostic**

**Enriched media**
- Used to grow particular organisms that might not be present in sufficient numbers to allow it be isolated and identified.
- Substances like blood, serum, egg are added to the defined medium.
- Eg: Blood agar, Chocolate agar (used for growing fastidious respiratory bacteria, such as *Haemophilus influenzae* and *Neisseria meningitidis*)