SACRAMENTO STATE
Leadership begins here.

Chapter 5 Metabolism: *Glycolysis & Fermentation*

The material presented in this lecture will be tested on Exam #2.

Exam #1 is Wednesday! Please bring a Scantron form.

Dr. Amy Rogers

Office Hours: Mondays & Wednesdays 9-10 AM
Sequoia 530

Some figures taken from Krogh Biology: A Guide to the Natural World

Bacterial Metabolism: 3 pathways to extract energy from glucose

- Glycolysis
- Fermentation
- Aerobic Respiration

(Krebs cycle, electron transport, oxidative phosphorylation)

Glycolysis

- *Ancient* metabolic pathway: 3.5 BYA…before earth's atmosphere had oxygen in it
- The first steps of energy extraction from glucose
- Does NOT require oxygen
- Autotrophs & heterotrophs, aerobes & anaerobes all do it
- Net energy yield is small: 2 ATP per glucose

Glucose is a 6 carbon sugar

Products of glycolysis:

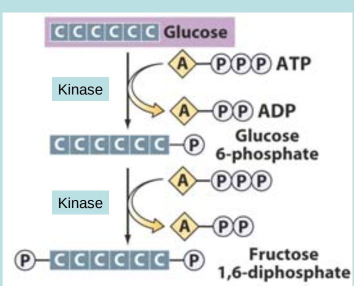
•Two 3-carbon molecules: **pyruvic acid** (x2)

•2 reduced **NADH** electron carriers: from 2 NAD+

•2 (net) **ATP**: from 2 ADP + 2 P_i

Glycolysis 1: **Substrate level phosphorylation**

1. Two phosphates from ATP are added to each glucose molecule
2. Glucose is isomerized into another 6 carbon sugar, **fructose**



Net energy yield: -2 ATP (2 ATP have been *consumed* to ADP + P_i)

Phosphate transfers are common

Kinase: generic name for any **enzyme** that *adds* a phosphate group to something

Phosphatase: generic name for any **enzyme** that *cleaves* a phosphate group from something

{These are *opposite* activities}

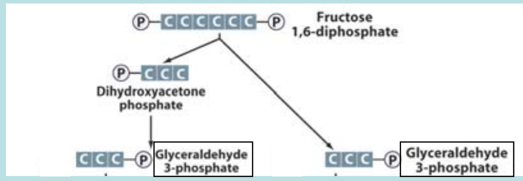
Why does it take ATP to make ATP?

1. Phosphorylation of glucose "raises its energy level so it can participate in subsequent reactions (like the rock pushed out of the depression atop the hill)."
2. Phosphorylated sugars are trapped inside the cell (plain glucose freely moves in & out)

Glycolysis 2: **Splitting**

- 6 carbon sugar (fructose) is split into two 3-carbon molecules
- Each molecule gets one of the phosphate groups
- The molecules are *not* identical

- One molecule is isomerized (rearranged) so the **two** 3-carbon molecules become **identical**: **glyceraldehyde 3-phosphate**



Glycolysis 3: Rearrangements & energy capture

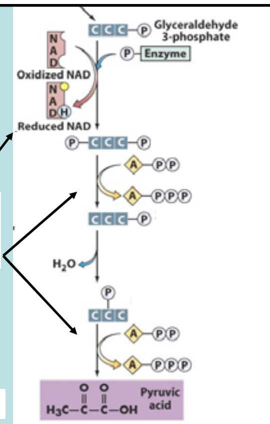
- In this series of enzyme-catalyzed reactions, *energy* is first extracted from the food (glucose)

★ The energy is captured in two forms:
  - NAD+ is reduced to NADH (one per 3 carbon unit) Carries energy as "reducing power" (more on this later)
  - ADP+P_i ➡ ATP (two per 3 carbon unit) (Substrate-level phosphorylation)
    - » The inorganic phosphate (P_i) comes from the phosphorylated 3 carbon units

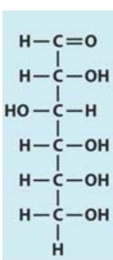
Remember: All this happens x2 per glucose as each 6 carbon glucose yields two 3 carbon G3P molecules

1. NAD+ reduced to NADH

2. ATP produced from ADP+P_i

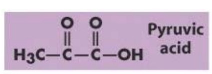


★ End product: **pyruvic acid** (x2)



Glucose ($C_6H_{12}O_6$)

Note how the products of glycolysis (pyruvic acid, or pyruvate) are ***oxidized*** relative to the initial substrate (glucose)



Carbo loading & glycolysis

- Each reaction in glycolysis is catalyzed by an enzyme
- Enzyme activity is usually regulated in some way
- Part of the reason why **"Carbo loading"** (eating a great deal of carbohydrates before an athletic endurance event) works may be that it *induces expression* of glycolytic enzymes.
  - More enzyme = faster catalytic activity = faster glycolysis

The energy balance sheet for Glycolysis

1 Glucose

(-2 ATP)

2 G3P

+2 NADH (1 per G3P)
+2 ATP
+2 ATP

2 pyruvate — Net products of glycolysis: 2 ATP, 2 NADH ★

Bacteria are highly diverse in their metabolic processes.

In certain species, other pathways exist for breaking down glucose & other sugars.

★ **Bacteria can only perform a reaction if they produce the proper enzyme** (for a pathway like glycolysis, a different enzyme for each step)

★ Ability to produce an enzyme is **genetically determined** and **species-specific**

Energy acquisition from *other* sugars:

Frequently, specific enzymes are used to convert the sugar ***into an intermediate of the glycolytic pathway*** ★
(one of those 3 carbon molecules…)

What gets consumed during glycolysis?

- Glucose
- ADP + P_i
- NAD+

Without a fresh supply of these reagents, glycolysis will stop.
Get more:
•Glucose: eat something; in mammals, mobilize glycogen reserves
•ADP: Burn energy (convert ATP to ADP + P_i)

????? NAD+ ?????

Oxidation of NADH to regenerate NAD+ for glycolysis

NADH is reduced; needs to pass off its electrons to another electron acceptor to regenerate oxidized NAD+

- In the presence of oxygen:

  Electron transport chain ★
  (***oxygen*** is the terminal electron acceptor)

- In the absence of oxygen:

  Fermentation ★
  (***organic*** terminal electron acceptors)

Fermentation

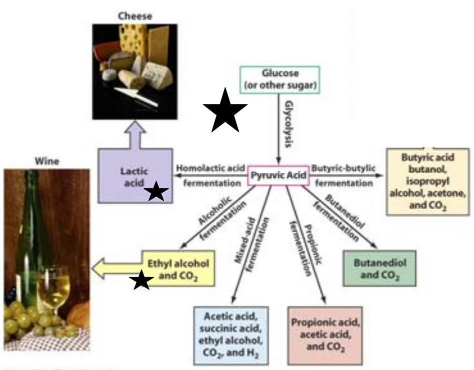


Figure 5-12 Microbiology, 6/e

★ Fermentation

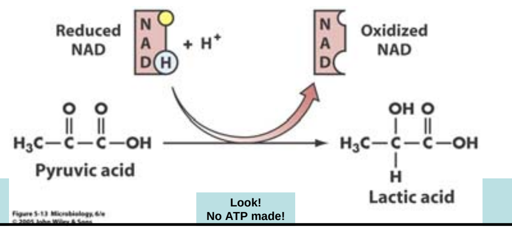
- **Species-specific metabolic pathways for reducing pyruvic acid in the <u>absence</u> of oxygen**
- Some species can ferment sugars *other* than glucose
- End products of fermentation tell you which pathway was used

NADH is oxidized back to NAD+, allowing glycolysis to continue

Energy is <u>NOT</u> captured by (most) **fermentation reactions!**

★ Homolactic acid fermentation

- Simplest pathway, one step conversion of pyruvic acid
- Only one (*homo*-) product: lactic acid. **No gas produced**
- **Lactobacilli (**some **cheeses); streptococci**; also mammalian muscle cells

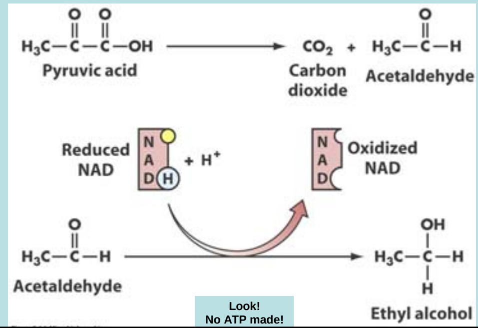


Look!
No ATP made!

Figure 5-13 Microbiology, 6/e
© 2005 John Wiley & Sons

★ Alcoholic Fermentation

- 2 steps; **CO_2** gas & **ethyl acohol** are products
- Rare in bacteria, common in yeast
- Bread & wine



Look!
No ATP made!

Other fermentation pathways

- Performed by a great variety of microbes
- We'll test for many pathways in lab
  - generally by looking for end products or intermediates
- A huge range of products can be produced
- Many have commercial utility; others are involved in disease, food spoilage, etc.

★Terminal electron acceptors

- The goal of fermentation is to oxidize NADH
- Something must be reduced (the electrons must go somewhere)
- Organic compounds (for example, lactic acid & ethyl alcohol) are the terminal electron acceptors in fermentation pathways

Later: how using **OXYGEN** as the terminal electron acceptor is a MUCH better deal!!!

Just to remind me to tell you: Table 6.6 has a discussion of the TSI test

TABLE 6.6

Selected Examples of Diagnostic Media

Medium	Organism(s) Identified	Selectivity and/or Differentiation Achieved
Triple sugar-iron agar (TSI)	Gram-negative enterics	*Not Selective* *Differential* Used in agar slants (tubes cooled in slanted position), where differentiation is based on both aerobic surface growth (slant) and anaerobic growth in agar in base of tube (butt). Medium contains specific amounts of glucose, sucrose, and lactose, sulfur-containing amino acids, iron, and a pH indicator, so relative use of each sugar and H_2S formation can be detected. 1. Uninoculated tube of TSI. 2. Inoculated: red slant and red butt = no change; no sugar fermented. 3. Yellow slant and yellow butt = lactose and glucose fermented to acid; trapped bubbles in butt indicate fermentation to acid and gas. 4. Red slant (lactose not fermented) and yellow butt (glucose fermented to acid); black precipitate = H_2S produced; sometimes obscures yellow butt. Almost all enteric pathogens produce red slant and yellow butt, with or without H_2S and/or gas.

Differentiation of Intestinal Bacilli Based on TSI

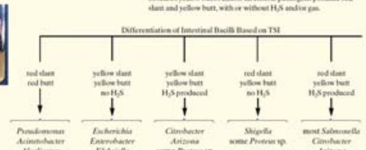


Table 6-6 part 2 Microbiology, 6/e