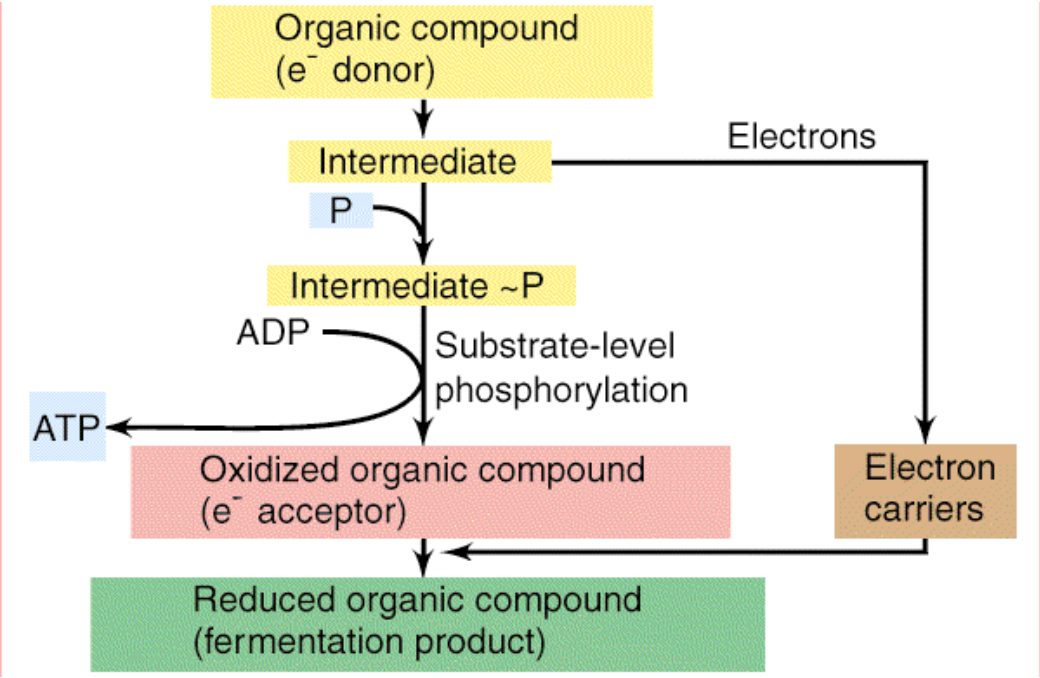
MMG 301, Lecture 19 Fermentation

Questions for today:

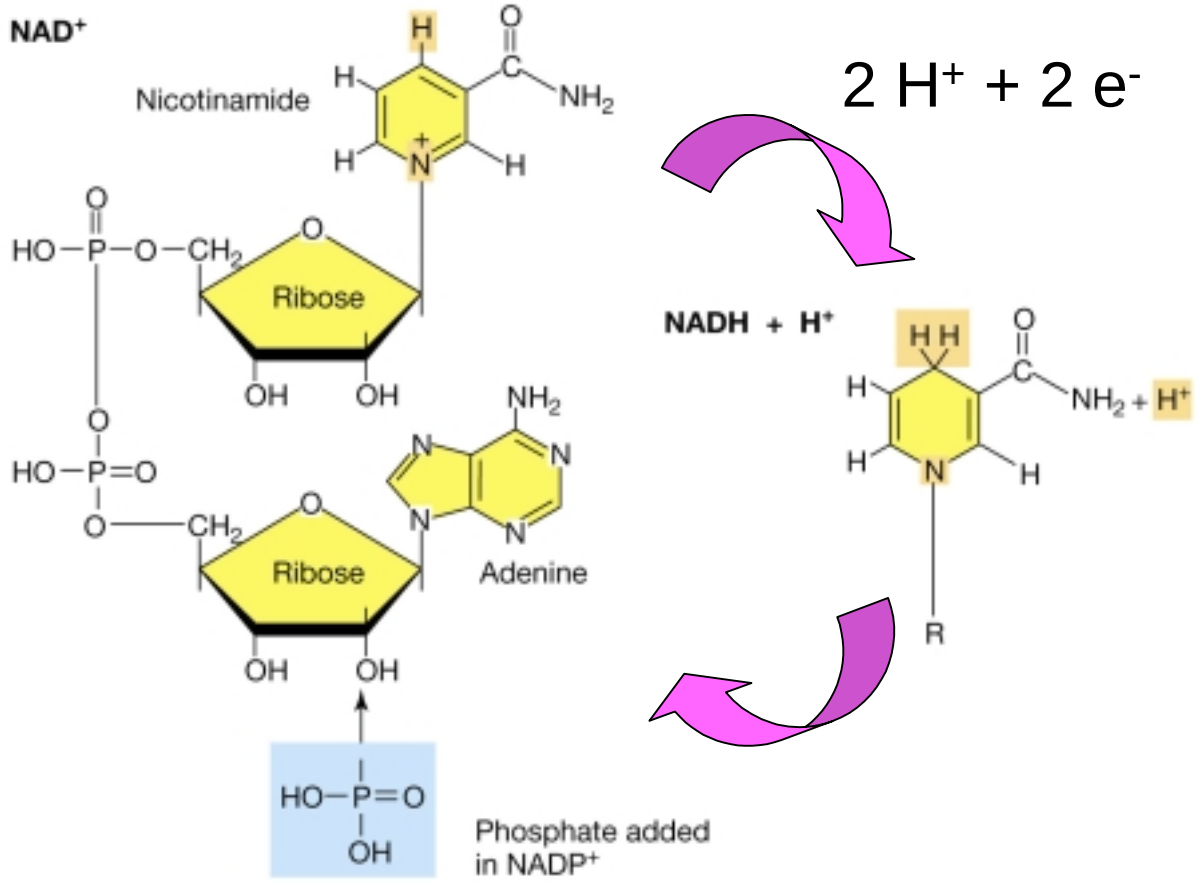
1. What is Fermentation?
2. What do we mean by Substrate Level Phosphorylation (SLP)?
3. What is the best-known fermentation pathway?
4. What are other types of fermentations?
5. How do I calculate the available energy?

Overview of Fermentation

Key features: Electrons exit the substrate via a carrier, then later return to form the product. Substrate is converted to a phosphorylated intermediate that allows for SLP



Major electron carrier: NAD(P)H



How can I keep track of oxidations and reductions?:

Lose	**G**ain
Electrons	**E**lectrons
Oxidized	**R**educed

(mnemonic device)

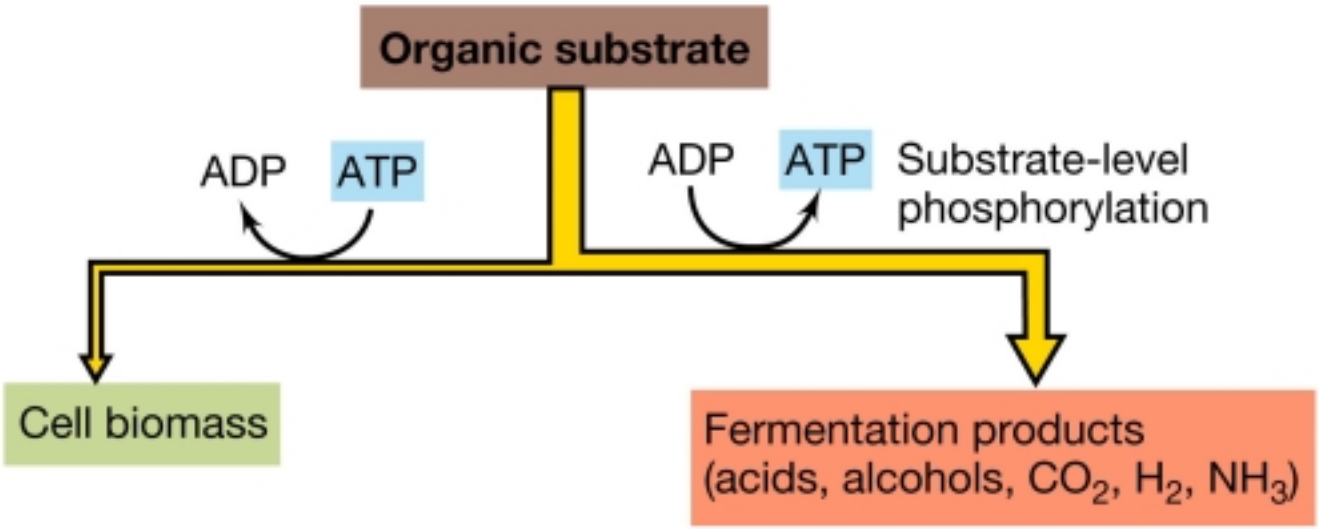
An easy method to assess the reduction state of organic compounds is to determine the H/O ratio

The H/O ratio: **Smaller # is more oxidized**
Larger # more reduced

Compound	Formula	H/O ratio
Carbon dioxide	CO_2	0
Glucose	$C_6H_{12}O_6$	2
Methane	CH_4	infinite
Lactic acid	CH_3-CH(OH)-COOH	2
Pyruvic acid	**CH_3-CO-COOH**	**1.33**
Ethanol	CH_3-CH_2-OH	?
Acetic acid	CH_3-COOH	?
2,3-butanediol	CH_3-CH(OH)-CH(OH)-CH_3	?

In fermentations the H/O ratio of the substrate(s) must match the H/O ratio of the product(s)!

What is the purpose of fermentation?



Energy Production by SLP

What is it?:

Energy-rich P donor X-P → ADP

(specific enzyme)

Y → ATP

Examples:

1,3-bisphosphoglycerate → 3-phosphoglycerate
+ ADP → ATP

phosphoenolpyruvate (PEP) → pyruvate
+ADP → ATP

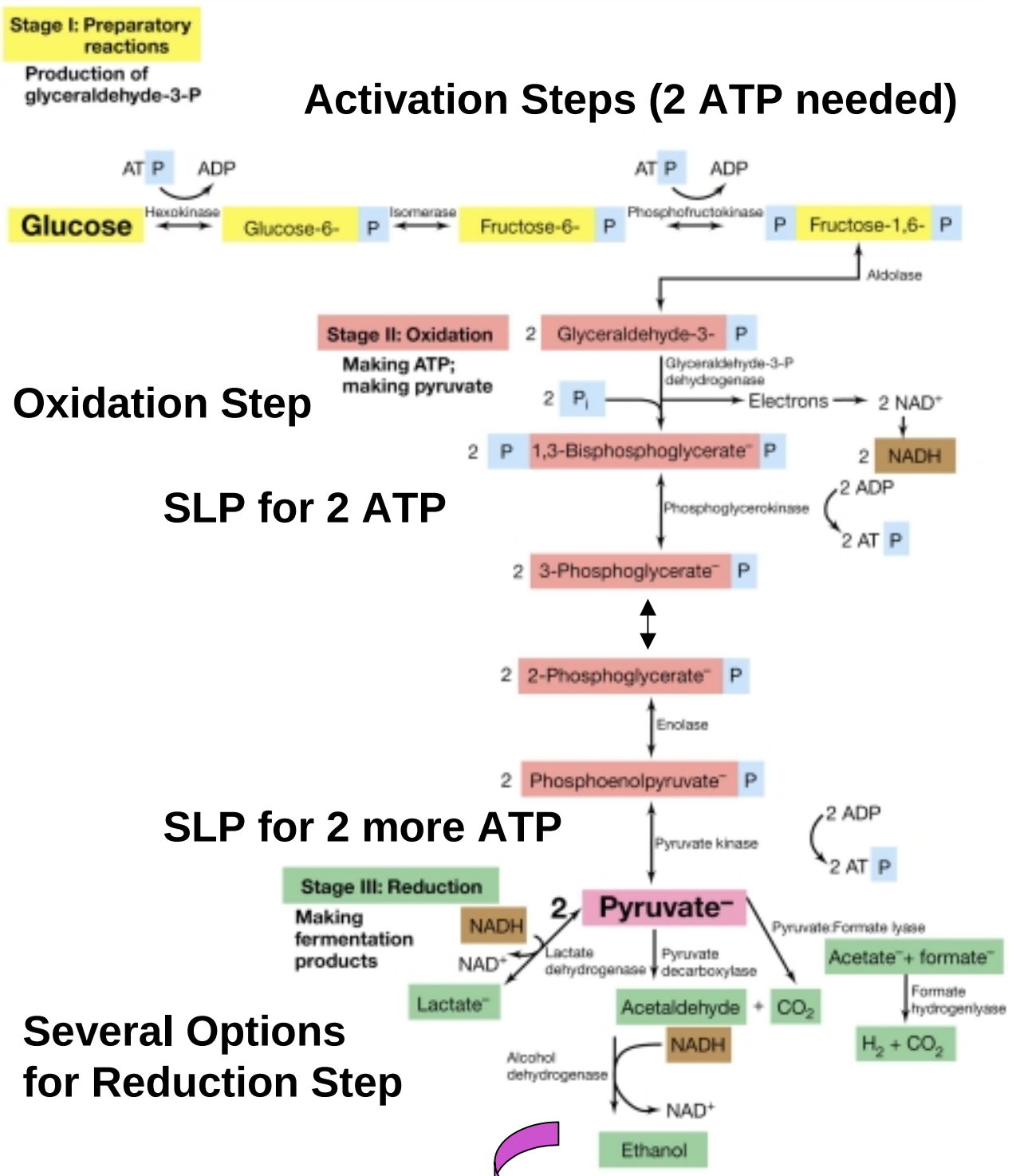
acetyl phosphate → acetate
+ADP → ATP

Membranes are not required or used.

Alcohol Fermentation by Yeast: A paradigm of fermentation

Glucose → 2 ethanol + 2 carbon dioxide

$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2$
(balanced H/O ratio!)



Glycolysis = conversion of glucose to 2 pyruvate
Acetaldehyde = CH_3CHO Ethanol = CH_3CH_2OH

Other Fermentations

Alcohol fermentation does occur in a few bacteria, but other types of fermentation pathways are much more common:

-- Alternative reactions of pyruvate
-- Alternative pathways from glucose to pyruvate
-- Alternative starting substrates
-- Named according to their final products

<u>Examples using glucose as the substrate</u>:

Lactic acid fermentations in Lactic Acid Bacteria

-- *Homofermentative*

glucose $\rightarrow$ 2 pyruvate $\rightarrow$ 2 lactic acid
(2 e^- ; 2 ATP)

-- *Heterofermentative*

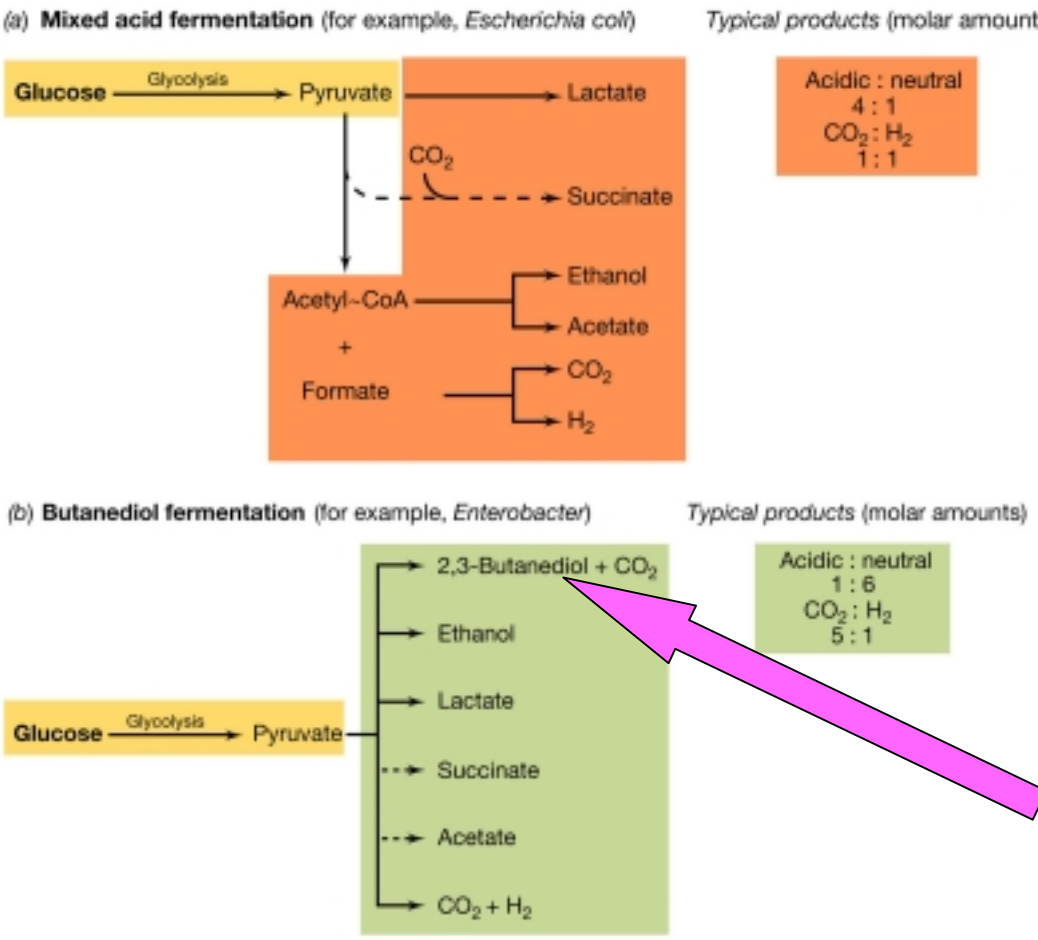
glucose $\rightarrow$ 2 pyruvate $\rightarrow$ lactic acid
ethanol
CO_2 (1 ATP)

These are distinguished by using analytical methods (e.g., GC, HPLC, measure gas production) to identify and quantify the products

Mixed acid fermentations

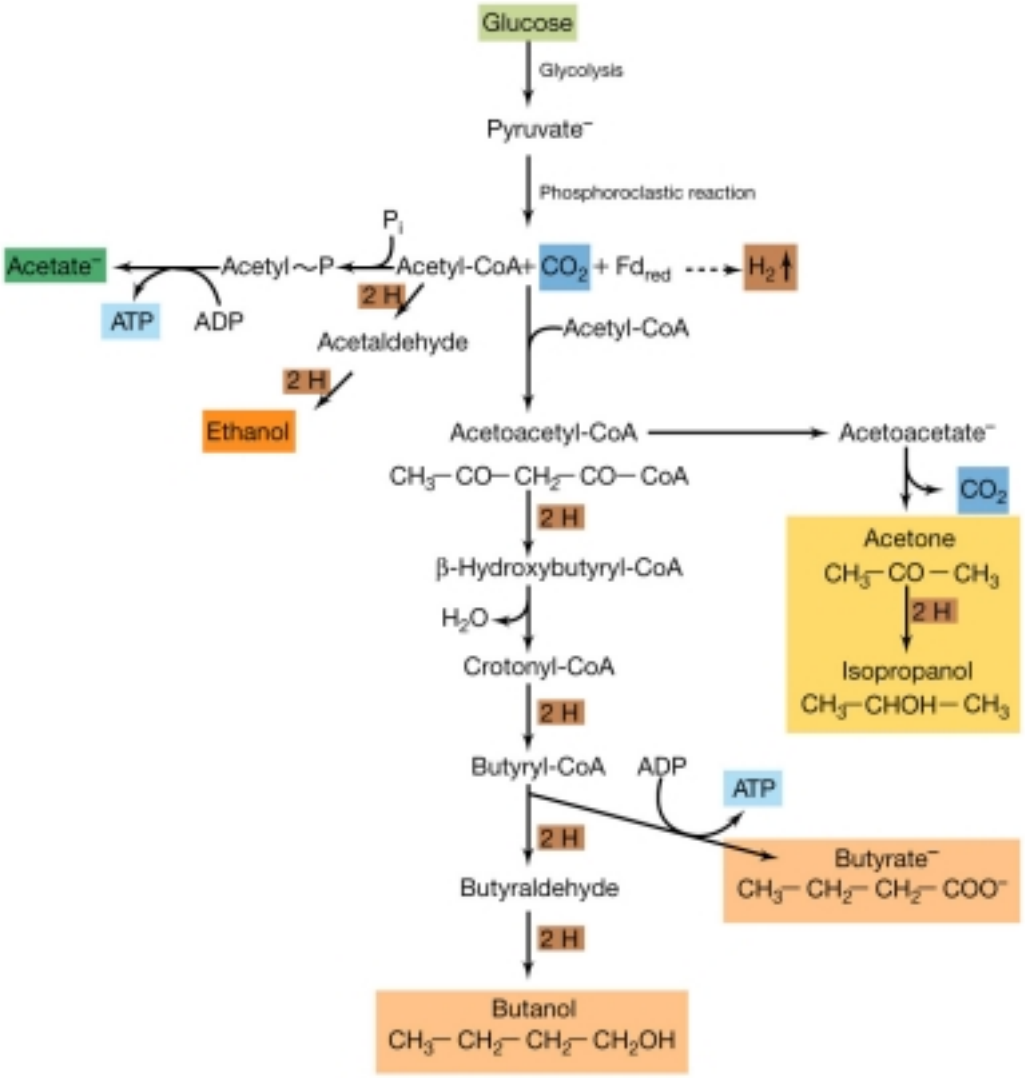
Butanediol fermentation

Other fermentations from glucose



Identify and quantify products by analytical methods (e.g., GC, HPLC, gas production) and simple chemical tests (e.g., Voges-Proskauer assay, pH indicators).

pH changes and gas production

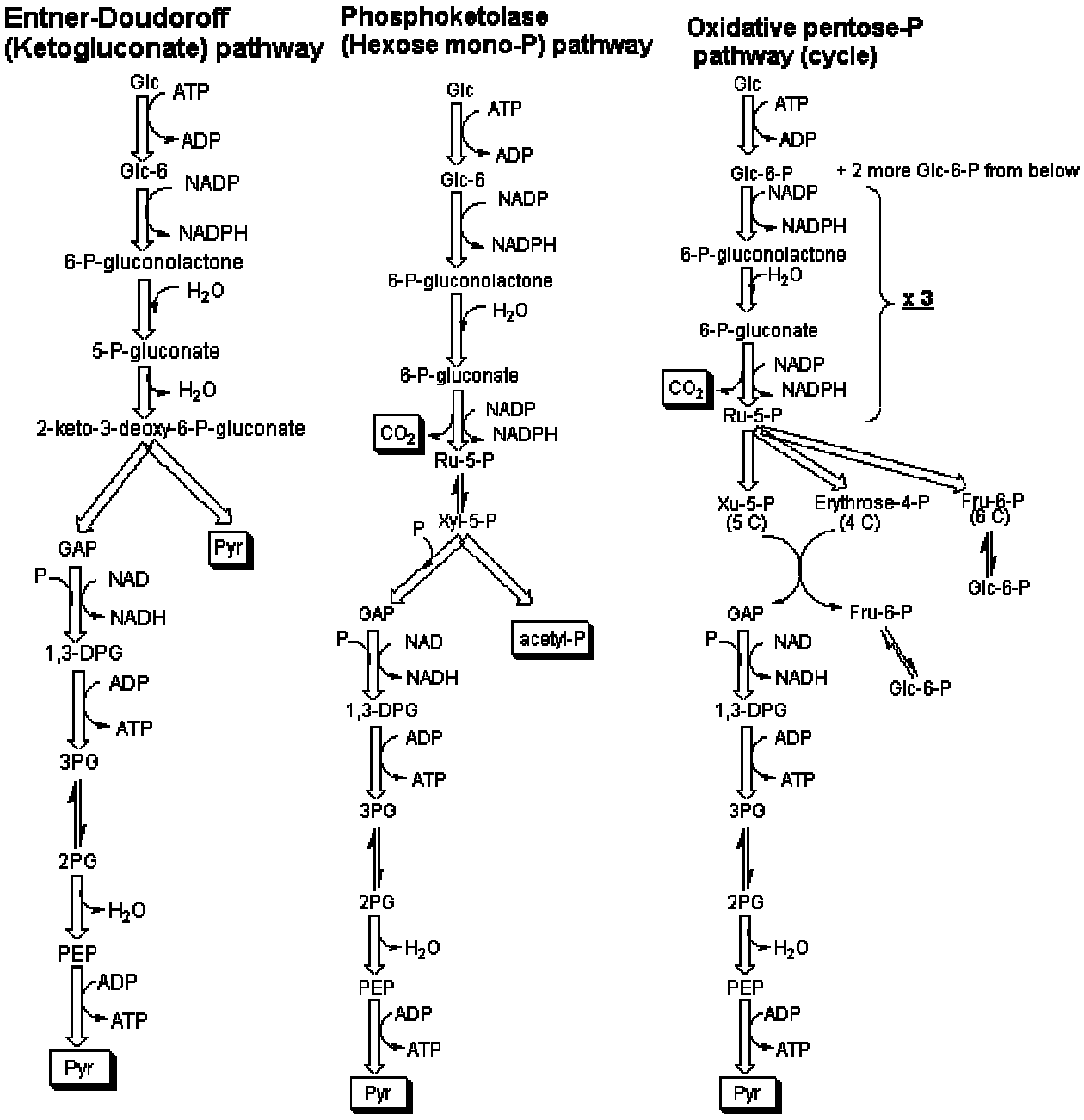


(Crucial for solvent production during WWII)

The identity of products produced helps to identify the microbe

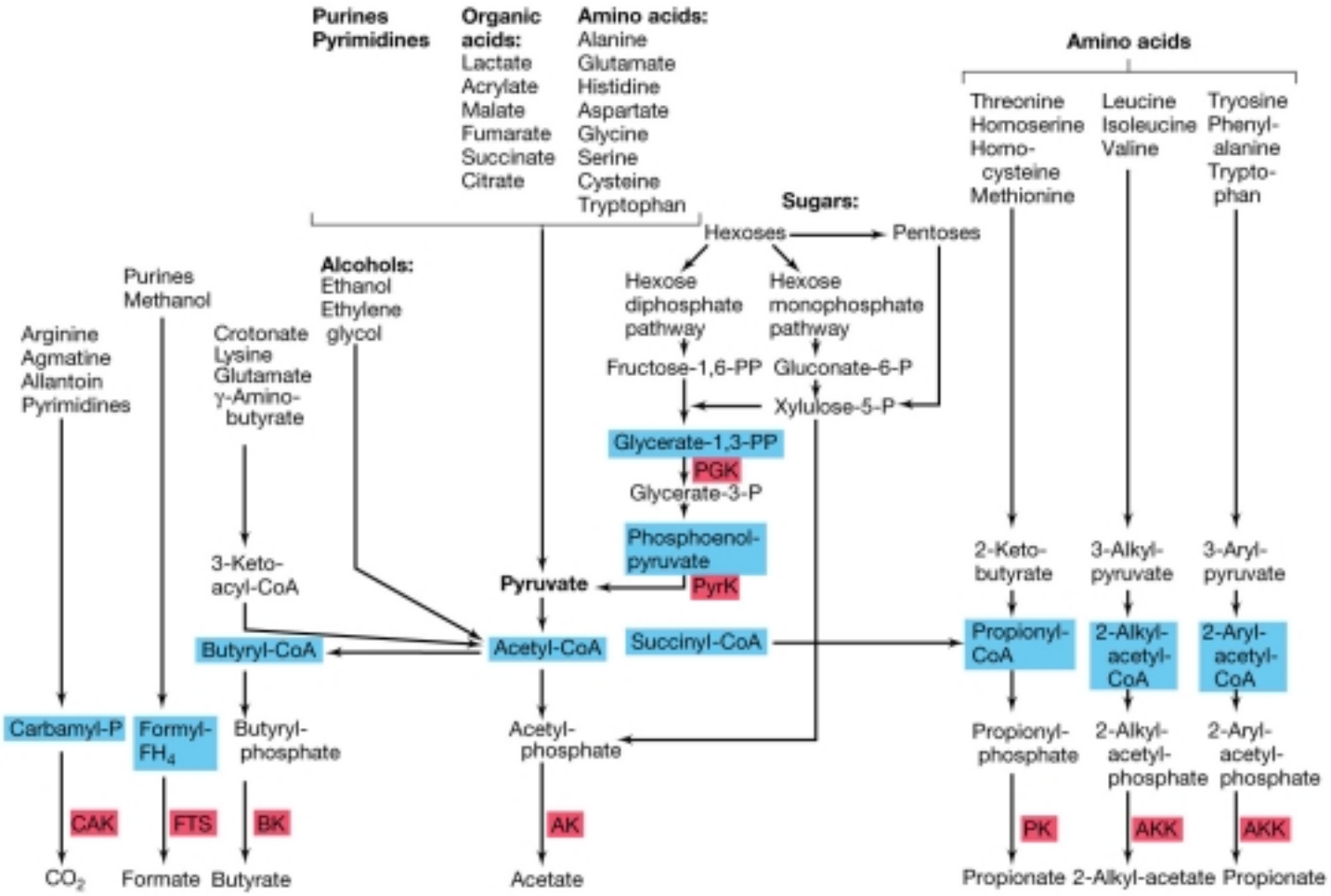
A few other paths from glucose to pyruvate

(don't memorize the details!)

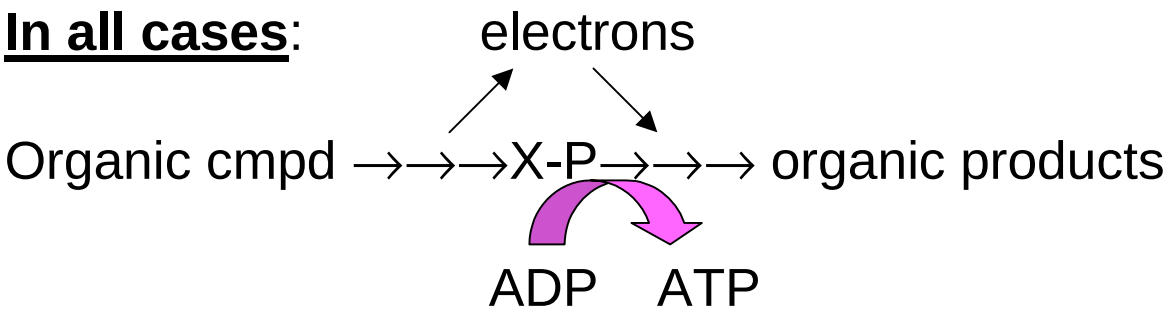


The pathway used by a particular microbe can be determined by measuring specific enzyme activities and testing for the presence of intermediates

Non-glucose fermentations



<u>In all cases</u>:

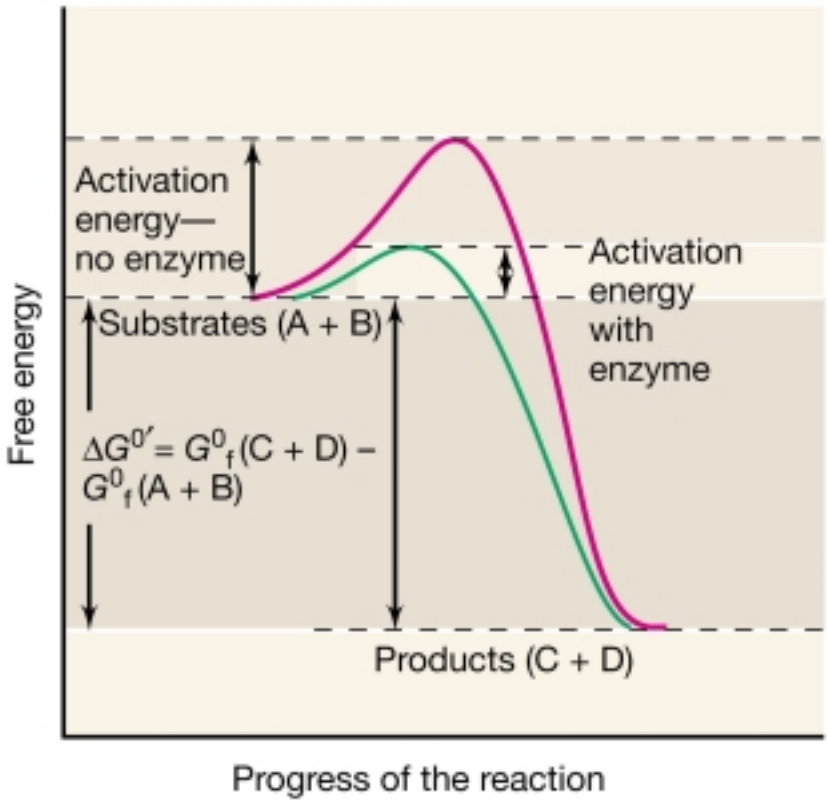


Types of substrates fermented can help to identify an unknown microorganism

How much energy is available?

$\Delta G^{o'}$ Gibb's free energy (measured in kJ/mole)

$\Delta G^{o'} = \Delta G^{o}_{f}$ (products) - ΔG^{o}_{f} (subtrates)



negative value = energy released
positive value = energy required

Table A1.1 lists *free energy of formation* (ΔG^{o}_{f}) for a range of compounds

Example: glucose → 2 ethanol + 2 CO_2

(-917 kJ/mol)(-182 kJ/mol)(-394 kJ/mol)

$\Delta G^{o'} = \Delta G^{o}_{f}$ (products) - ΔG^{o}_{f} (subtrates)

[2(-182) + 2(-394)] – (-917) = -235 kJ/mol

<u>What about ATP?</u>

$ATP + H_2O \rightarrow ADP + P_i$ $\quad\quad$ $\Delta G^{o'}$ = -32 kJ/mol

$P_i + ADP \rightarrow ATP + H_2O$ $\quad\quad$ $\Delta G^{o'}$ = 32 kJ/mol

Alcohol fermentation yields a net of 2 ATP

<u>Efficiency</u> = 100x(<u>energy in ATP generated</u>)

(energy available)

= 100x[2(-32 kJ/mol)]/(-235 kJ/mol)

= 27%

Other “high-energy phosphate donors” used in SLP:

	$\Delta G^{o'}$
1,3-bisphosphoglycerate	-52 kJ/mol
PEP	-51.6 kJ/mol
acetyl phosphate	-44.8 kJ/mol

This explains why these compounds can be used to make ATP

Things to think about regarding fermentation

- What controls whether a cell ferments glucose to ethanol or lactic acid?
- What controls whether a cell is capable of fermenting fatty acids vs sugars (and which types of sugars)?
- If a microbe can ferment either of two substrates, what controls the outcome if both substrates are present?
- How might pH affect the energetics of fermentation?