1.5: Intro to Microbial Metabolism

Microbial Metabolism: Bacterial Pathways

Oxygen (O_2) is essential for organisms growing by aerobic respiration (previous worksheet). Many organisms are unable to carry out aerobic respiration because of one or more of the following circumstances:

1. The cell lacks a sufficient amount of any final electron acceptor (such as O_2) to carry out cellular respiration.
2. The cell lacks genes to make appropriate complexes and electron carriers in the electron transport system (oxidative phosphorylation).
3. The cell lacks genes to make one or more enzymes in the TCA cycle.

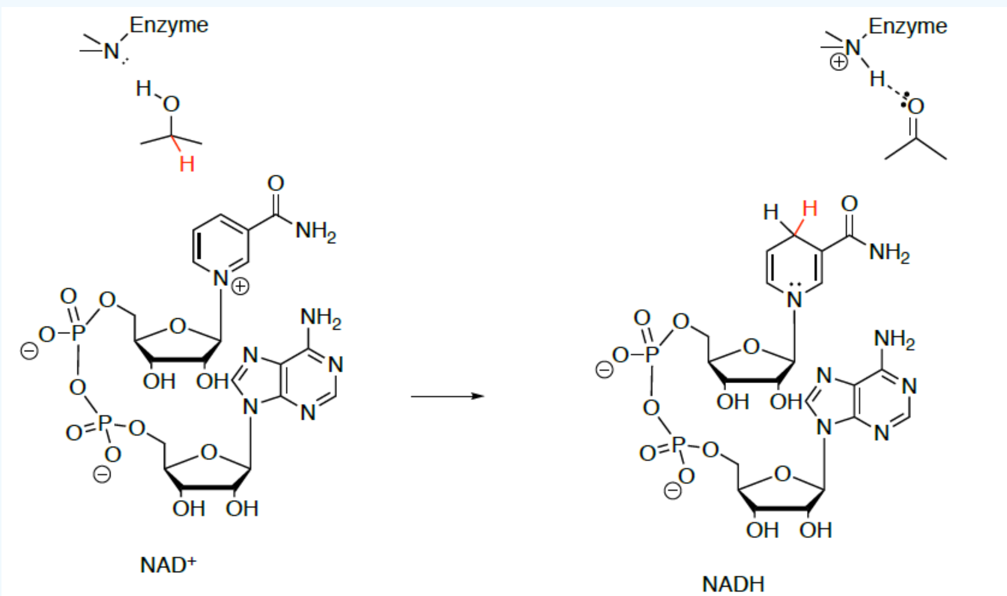
Fermentation usually refers to anaerobic processes in which organisms do not use molecular oxygen in respiration. Some microbes are *facultative fermenters*; they contain all the genes required to use either aerobic or anaerobic respiration pathways and they will use aerobic respiration unless there is no oxygen available. However, many prokaryotes are permanently incapable of respiration, even in the presence of oxygen because they lack enzymes or complexes to complete either TCA cycle or electron transport. These are *obligate anaerobes*.

Lactic Acid Fermentation

One important fermentation process is lactic acid fermentation. This process is common in lactobacilli bacteria (and many others). If respiration does not occur through oxidative phosphorylation, NADH must be re-oxidized to NAD^+ for reuse in glycolysis through the EMP pathway (covered earlier).

Exercise 1.5.1

- How many NAD^+ are created in glycolysis? _________
- Draw the arrows for the glycolysis reaction of NAD^+ to NADH.



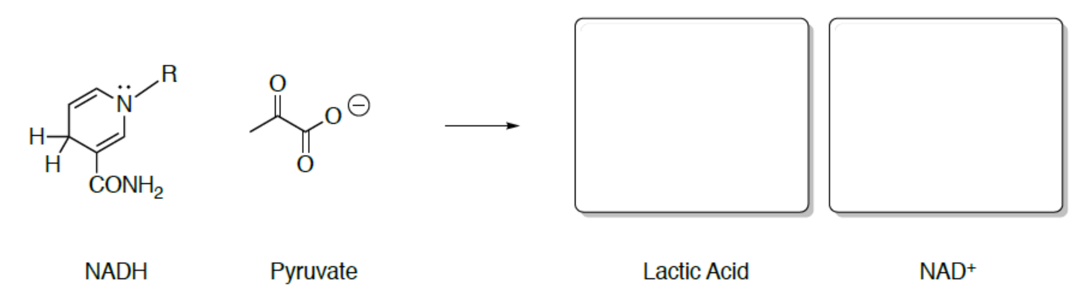
Exercise 1.5.2

NAD^+ is a catalyst in these reactions.

- Catalysts must change the activation energy. Normally the barrier to breaking a C-H bond is very high. How does this enzyme: NAD^+ complex achieve that?
- Catalysts must be regenerated. In aerobic metabolism, NADH is converted back to NAD+ by reacting with pyruvate (see below).

Facultative microbes, particularly bacteria, often use pyruvate as a final electron acceptor.

- Draw a curved arrow mechanism and predict products for this reaction.



Lactic acid fermentation regenerates NAD^+ but does not directly produce additional ATP.

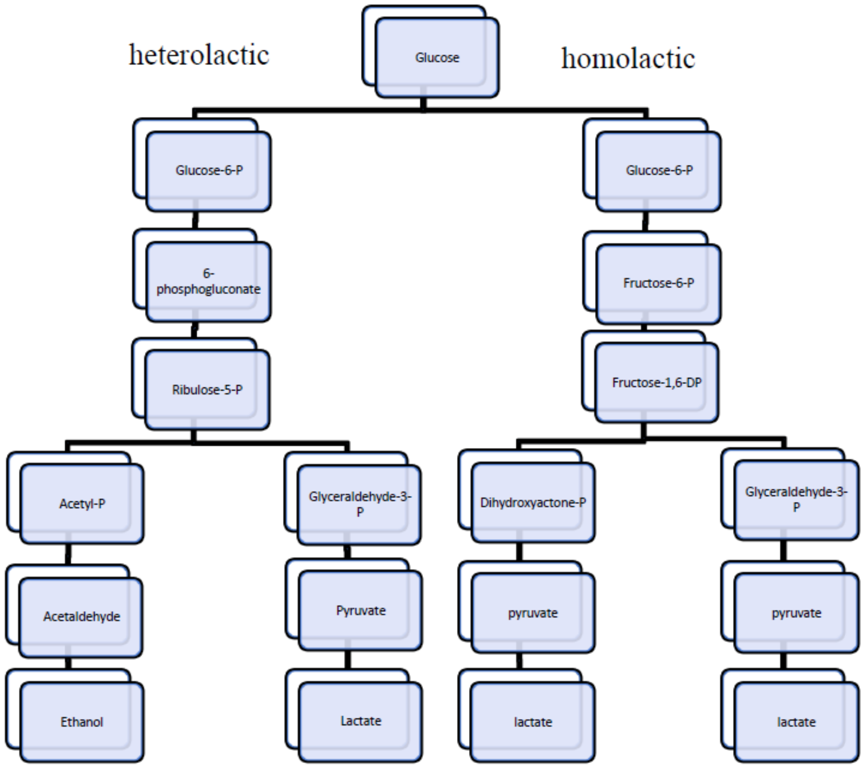
Exercise 1.5.3

- Thus, organisms carrying out fermentation produce a maximum of _____ ATP molecules per glucose during glycolysis.
- When there is sufficient O_2, facultative microbes will preferentially switch to cellular respiration for glucose metabolism. Explain why.
- Bacteria creating lactic acid as a side product, create a ____________ **[acidic / basic]** environment.
- The acidity of lactic acid impedes biological processes. This can be beneficial to the fermenting organism as it drives out competitors. Humans discovered that foods prepared with lactic acid fermentation will have a longer shelf-life. Explain in your own words.

Homolactic vs Heterolactic Fermentation

When lactic acid is the only fermentation product, the process is said to be ***homolactic fermentation***; such is the case for *Lactobacillus delbrueckii* and *S. thermophiles* used in yogurt production.

However, many bacteria perform ***heterolactic fermentation*** utilize the pentose phosphate pathway to produce a mixture of lactic acid and ethanol. More detail on this pathway follows. One important heterolactic fermenter is *Leuconostoc mesenteroides*, which is used for souring vegetables like cucumbers and cabbage, producing pickles and sauerkraut, respectively.



? Exercise 1.5.4

- Thus, organisms carrying out fermentation produce a maximum of _____ ATP molecules per glucose during glycolysis.
- When there is sufficient O_2, facultative microbes will preferentially switch to cellular respiration for glucose metabolism. Explain why.
- Bacteria creating lactic acid as a side product, create a ____________ **[acidic / basic]** environment.
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Pentose Phosphate Pathway

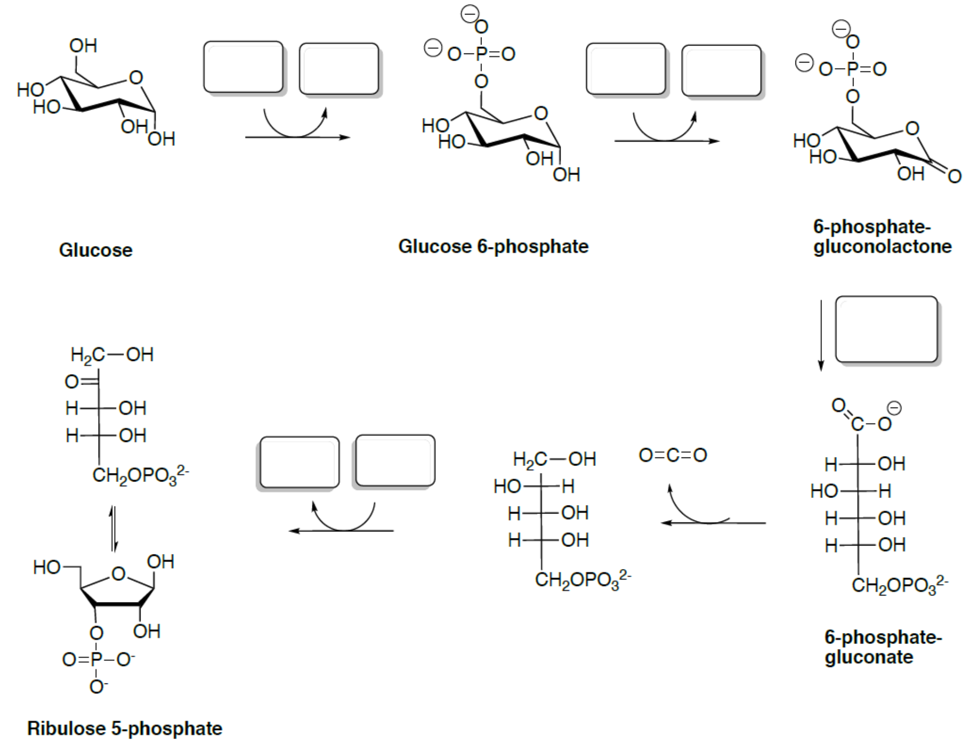
The pentose phosphate pathway has three primary roles in metabolism (human and prokaryotic).

1. Production of ribose 5-phosphate (R5P) for synthesis of nucleotides and nucleic acids.
2. Production of erythrose 4-phosphate (E4P) for synthesis of aromatic amino acids.
3. The PPP creates NADPH (up to 60% of NADPH production comes from this pathway).

There are two phases to these pathways: oxidative phase and non-oxidative phase.

? Exercise 1.5.5

- Add curved arrows and missing biological reagents to this schematic for the *oxidative phase* of pentose phosphate pathway

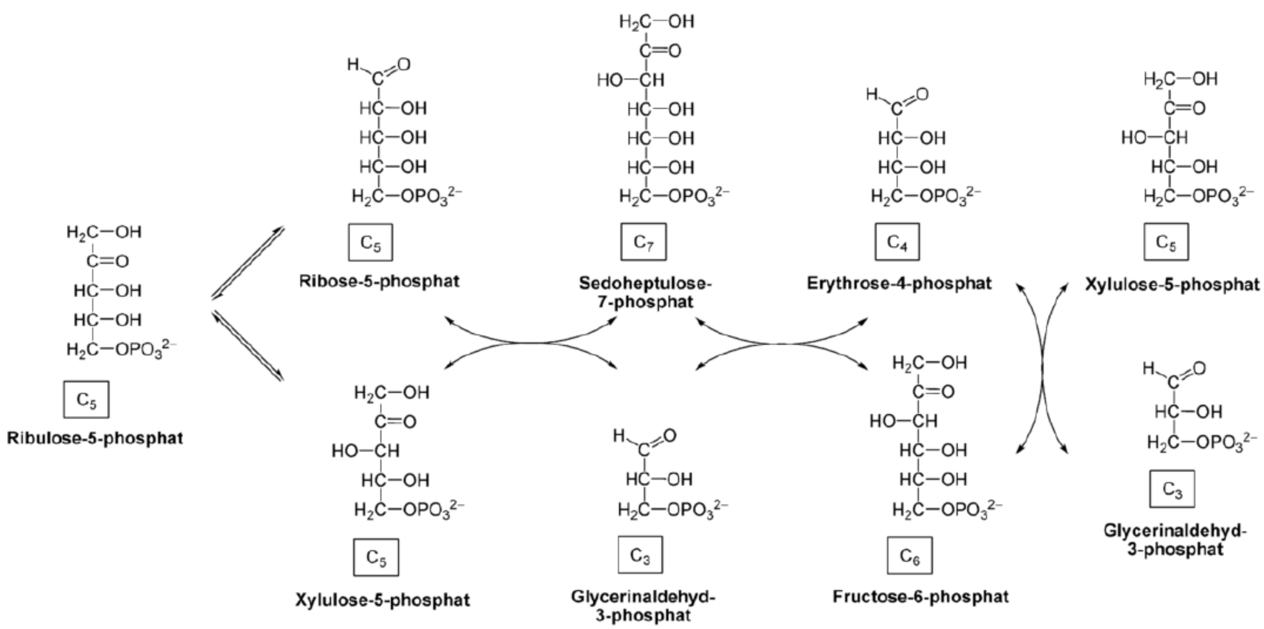


Ribulose-5-phosphate (the product of the oxidative stage) is the precursor to the sugar that makes up DNA and RNA.

Exercise 1.5.6

- How many NADPH are produced for each glucose in this phase of the pathway?

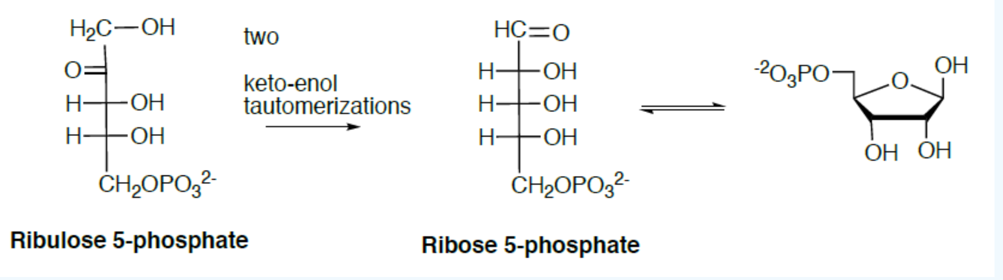
In the *non-oxidative phase*, there are different options that depend on the cell's needs. The ribose-5-phosphate from step 3 is combined with another molecule of ribose-5-phosphate to make one, 10-carbon molecule. Excess ribose-5-phosphate, which may not be needed for nucleotide biosynthesis, is converted into other sugars that can be used by the cell for metabolism.



Ribulose-5-phosphate (the product of the oxidative stage) is the precursor to the sugar that makes up DNA and RNA.

? Exercise 1.5.7

- Propose a mechanism for this conversion to the cyclic ribose-5-phosphate (three steps!).

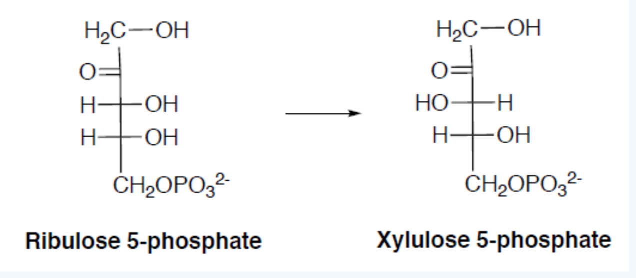


Of interest for heterolactic fermentation, ribose-5-phosphate is converted to glyceraldehyde-3- phosphate which enters the glycolysis pathway to be converted to pyruvate and then lactic acid.

The first step is a simple epimerization alpha to the carbonyl to convert ribose-5-phosphate to xylulose-5-phosphate.

? Exercise 1.5.8

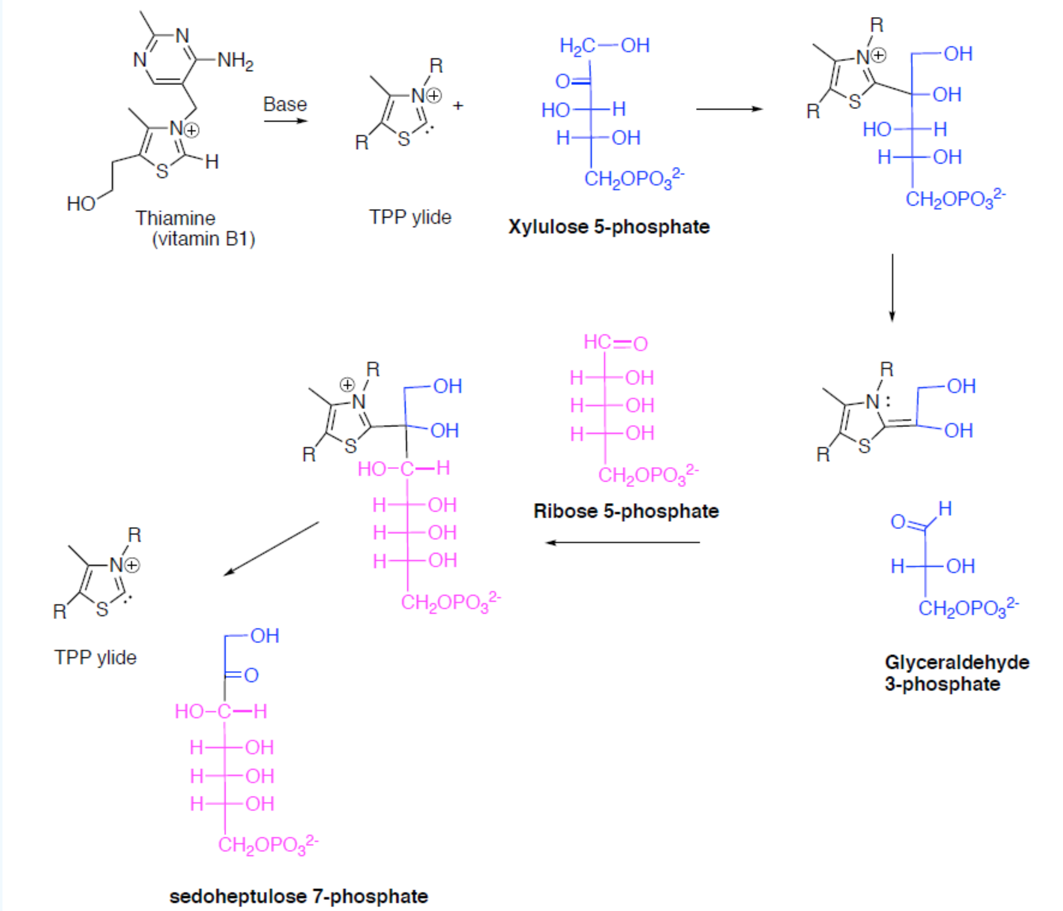
Propose a mechanism for this interconversion.



The second step is a reaction of xylulose-5-phosphate with a ribose-5-phosphate to prepare a 7- carbon sugar and the glyceraldehyde-3-phosphate.

? Exercise 1.5.9

Draw curved arrows for this mechanism.



The glyceraldehyde-3-phosphate is then converted to lactic acid. This is a repeat of glycolysis and homolactic acid fermentation.

? Exercise 1.5.10

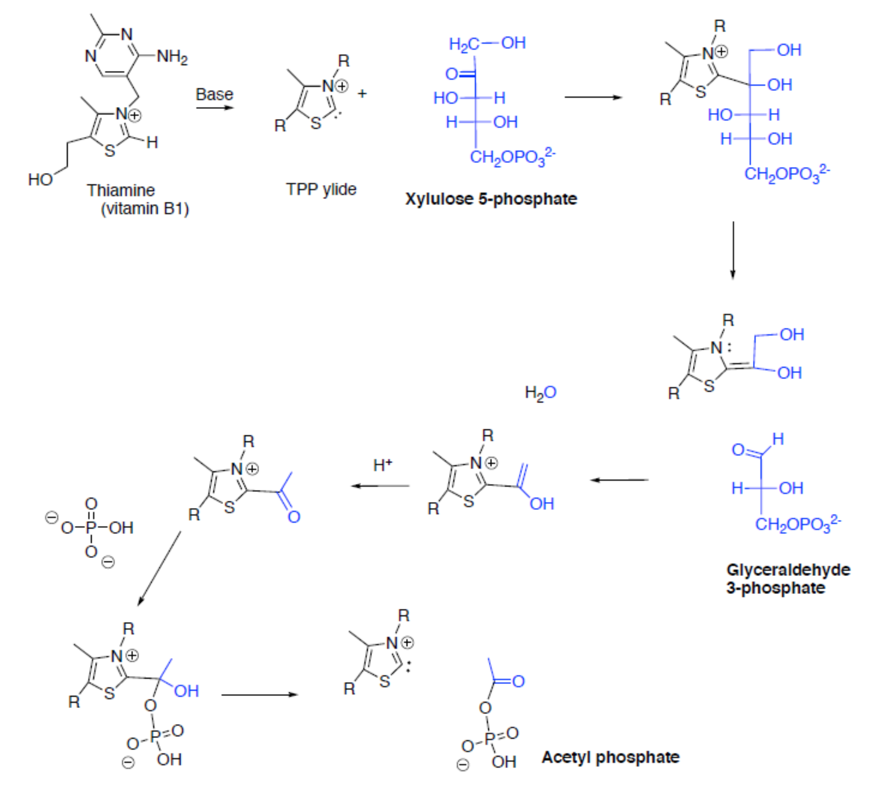
Draw out the pathway to convert glyceraldehyde-3-phosphate to lactic acid.

The next steps follow a similar pathway to produce other length sugars and more glyceraldehyde-3-phosphate.

In heterolactive fermentation, xylulose-5-phosphate can also be converted directly to glyceraldehyde-3-phosphate and acetyl phosphate.

? Exercise 1.5.11

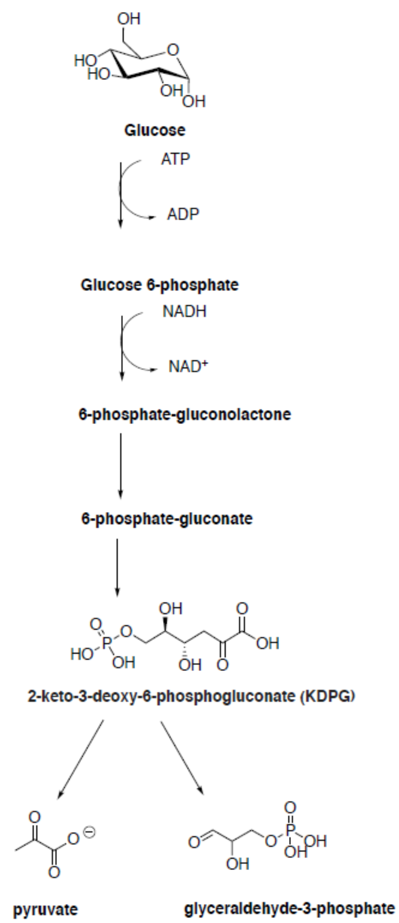
Draw curved arrows for this mechanism.

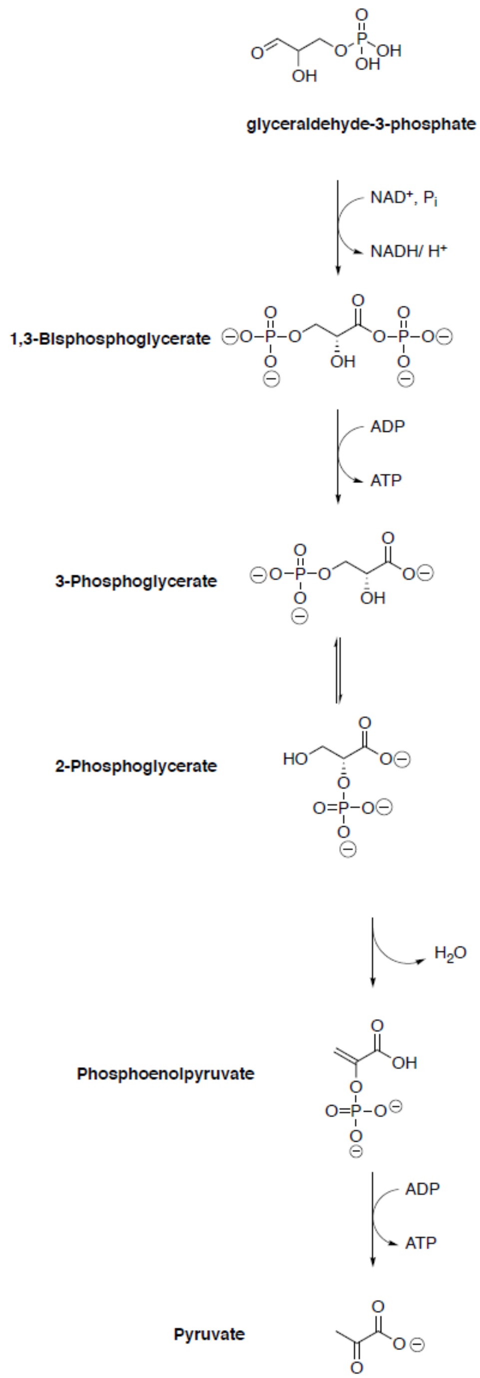


Acetyl phosphate can then be converted to ethanol. Suggest some steps for this conversion (HINT: Look at the ethanol fermentation pathway in yeast).

Entner-Doudoroff (ED) Glycolytic Pathway

Some bacteria often utilize the Entner-Doudoroff (ED) Glycolytic Pathway rather than the classic glycolysis pathway.


Glucose
ATP
ADP
Glucose 6-phosphate
NADH
NAD+
6-phosphate-gluconolactone
6-phosphate-gluconate
2-keto-3-deoxy-6-phosphogluconate (KDPG)
pyruvate
glyceraldehyde-3-phosphate



? Exercise 1.5.12

- How does the ED pathway differ from classic Embden-Meyerhof glycolysis pathway? Be specific.
- How does the ED pathway differ from pentose phosphate pathway? Be specific.
- The Entner–Doudoroff pathway also has a net yield of ________ ATP for every glucose molecule processed, as well as 1 NADH and 1 NADPH.
- Embden-Meyerhof glycolysis has a net yield of _________ ATP and ______ NADH for every glucose molecule processed.

Sources

du Toit, Englebrecht, Lerm, & Krieger-Weber, Lactobacillus: The Next Generation of Malolactic Acid Fermentation Starter Cultures, *Food Bioprocess. Technol*. **2011**, *4*, 876-906.