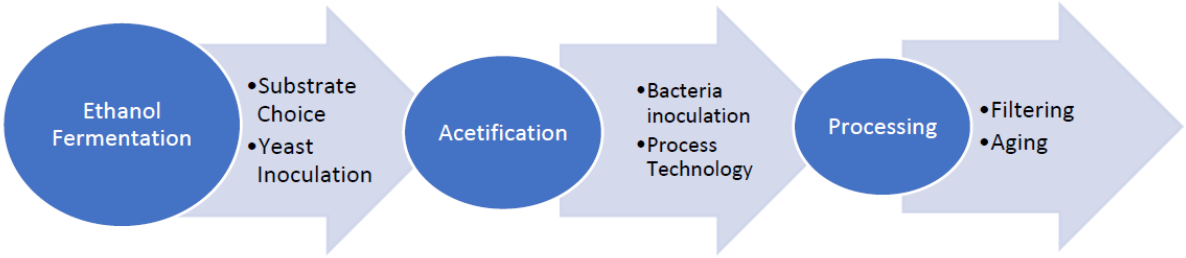
1.6: Vinegar and Acetic Acid Fermentation

Vinegar Production

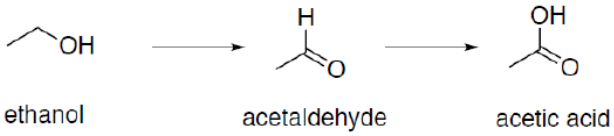


Acetic Acid Fermentation

The first description of microbial vinegar fermentation was made by Pasteur in 1862. He recognized that vinegar was produced by a living organism.

Overview of Acetic Acid Metabolism

Acetic acid bacteria (AAB), genus *Acetobacter*, are a group of Gram-negative bacteria which oxidize sugars or ethanol and produce acetic acid during fermentation. There are several different genera in the family Acetobacteraceae. AAB are found in sugary, alcoholic and acidic niches such as fruits, flowers and particularly fermented beverages. Given sufficient oxygen, these bacteria produce acetic acid (vinegar) from ethanol.



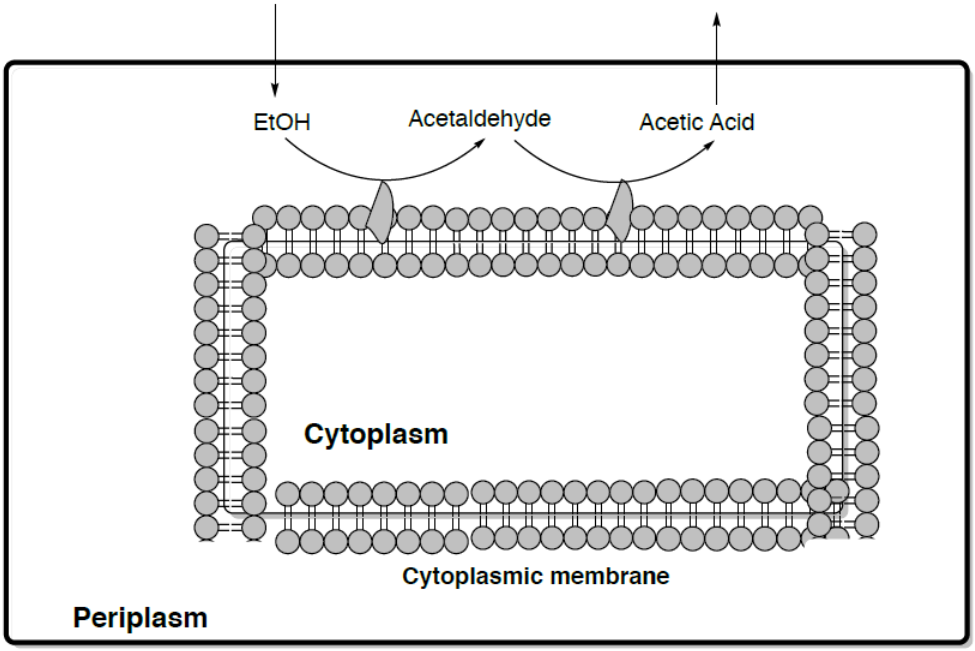
Several species of acetic acid bacteria are used in industry for production of certain foods and chemicals. Commonly used feeds include apple cider, wine and fermented grain mashes. AAB are also involved in the production of other foods such as cocoa powder and kombucha. However, they can also be considered spoilage organisms.

? Exercise 1.6.1

List 2-3 places/times that acetic acid bacteria would be considered spoilage organisms.

Location of Ethanol Oxidations

AAB make acetic acid by two successive catalytic reactions of the alcohol dehydrogenase (ADH) and a membrane-bound aldehyde dehydrogenase (ALDH) that are bound to the periplasmic side of the cytoplasmic membrane.

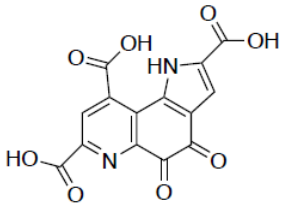


Ethanol, acetaldehyde, and acetic acid can be quite toxic for living organisms. However, AAB are able to live in both alcoholic and acid media because of a few adaptations.

1. Location of the alcohol dehydrogenases. Are the toxic compounds ever entering the cell cytoplasm?
2. The ALDH and ADH is one complex in many AAB species, thus never releasing acetaldehyde.
3. Acetobacter have H^+ pumps that actively remove protons from the cells.
4. There are changes in the composition of membrane phospholipids to help maintain membrane fluidity at low pH.
5. Many cellular proteins show increased negative surface charge that stabilizes them at low pH.

Location of Acetic Acid Metabolism with PQQ

AAB are able to oxidize ethanol to acetic acid using a membranebound ADH and ALDH complexes with a PQQ cofactor.



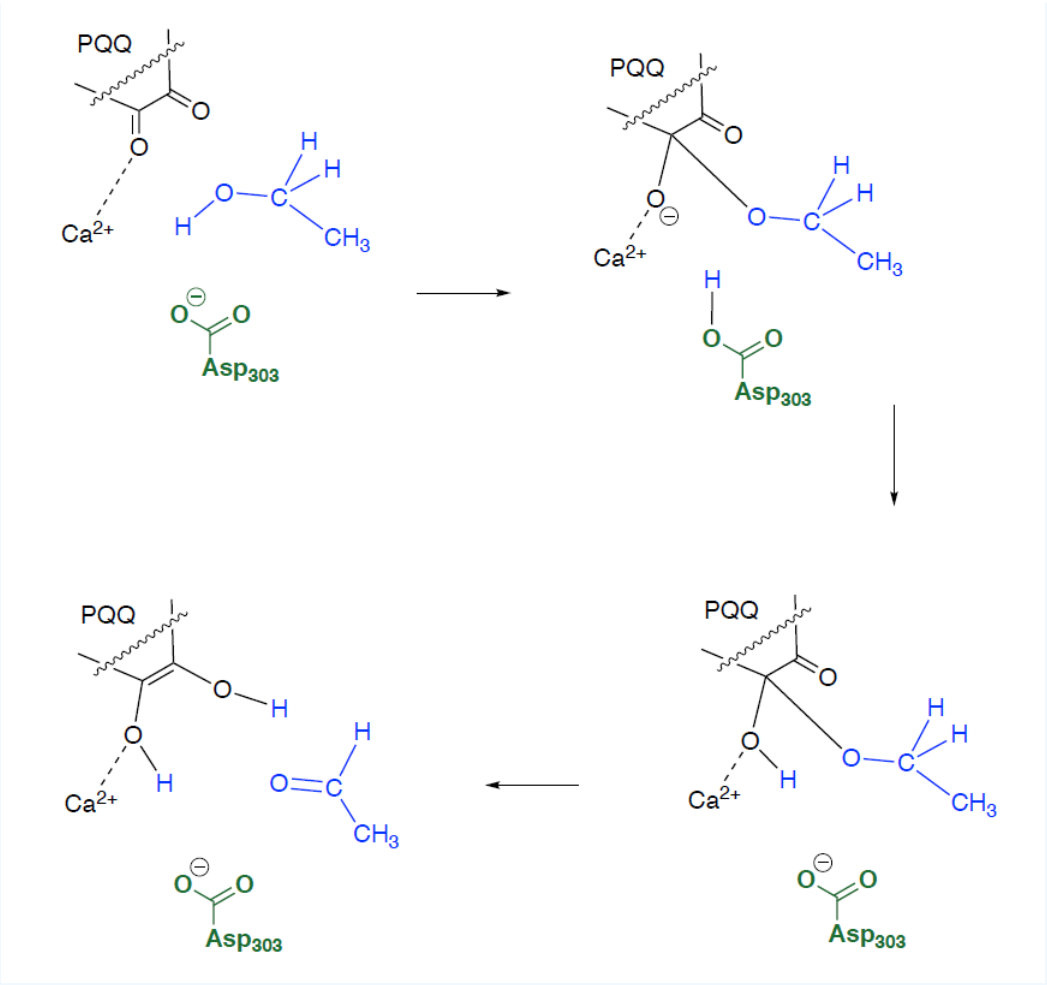
PQQ cofactor

This enzyme is capable of oxidizing a few primary alcohols (C2 to C6) but not methanol or secondary alcohols.

PQQ Reaction Mechanisms:

? Exercise 1.6.2

Add a curved arrow mechanism for the oxidation of ethanol to acetaldehyde using this PQQ cofactor.

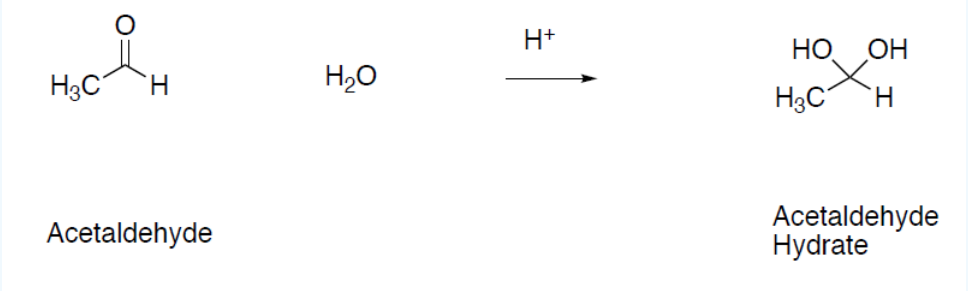


How many electrons are transferred from the ethanol molecule to the PQQ in this step?

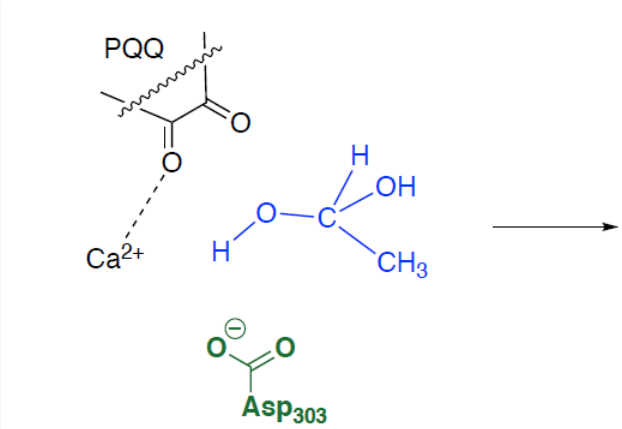
PQQ Reaction Mechanisms

? Exercise 1.6.3

In the second step, acetaldehyde forms a hydrate. Show the mechanism for this step.



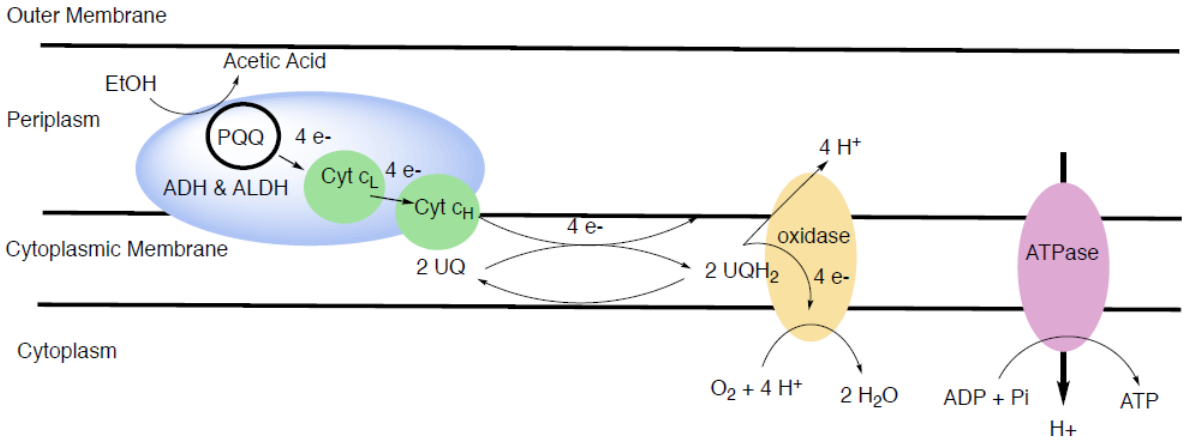
The acetaldehyde hydrate then reacts with another PQQ to form acetic acid. Propose a curved arrow mechanism for this transformation.



- How many electrons are transferred from the acetaldehyde hydrate molecule to the PQQ molecule?
- How many total electrons are involved in this two-step transformation?

PQQ tied to Electron Transport Process

The electrons are transferred electrons to ubiquinone (UQ) that are tightly linked to the respiratory chain (oxidative phosphorylation).



Exercise 1.6.4

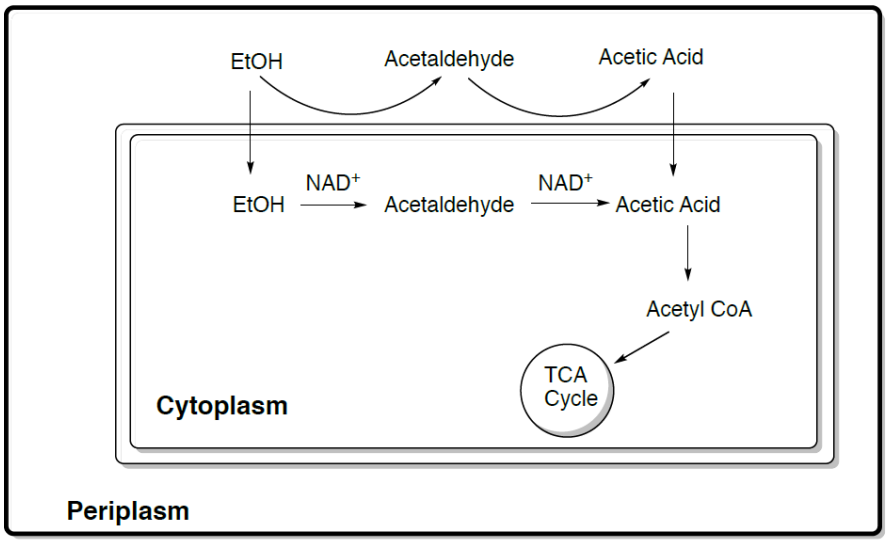
- For every EtOH molecule that is oxidized twice to Acetic Acid:

1. How many electrons move through the electron transport chain?
2. How oxygen atoms are converted to water?
3. How many protons are pumped across the membrane?
4. Assuming that approximately 3-4 protons yield 1 ATP, how many ATP produced?

- Why is this process considered to require oxygen? i.e. Why is this organism an obligate aerobe?
- What is the purpose of converting ethanol to acetic acid for these bacteria?

Acetic Acid Assimilation

Some *Acetobacter* and *Gluconacetobacter* strains can metabolize acetic acid to carbon dioxide and water using Krebs cycle enzymes. In vinegar, for instance, Acetobacter species exhibits a biphasic growth curve, where the first corresponds to an EtOH oxidation with AcOH production. The second spike in growth is due to '**acetic acid assimilation**' wherein the bacteria move the ethanol and/or acetic acid into the cytoplasm to metabolize using the TCA cycle and oxidative phosphorylation.



? Exercise 1.6.5

- What is advantage of using acetic acid assimilation?
- Why do the bacteria not use this pathway from the beginning?
- In vinegar fermentation, producers attempt to prevent this process. Explain why.

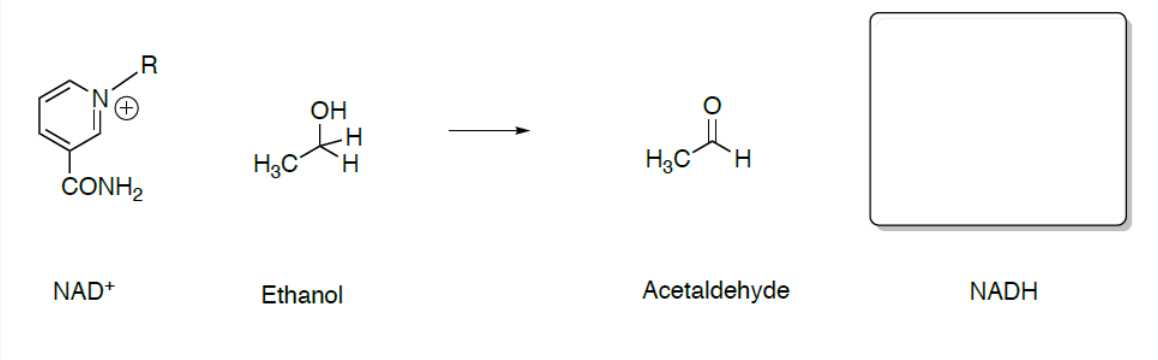
Mechanisms of NAD^+ Driven Dehydrogenases in Acetobacter

The overall chemical reaction facilitated by these bacteria is:

$$C_2H_5OH + O_2 \rightarrow CH_3COH \rightarrow CH_3COOH + H_2O$$

? Exercise 1.6.6

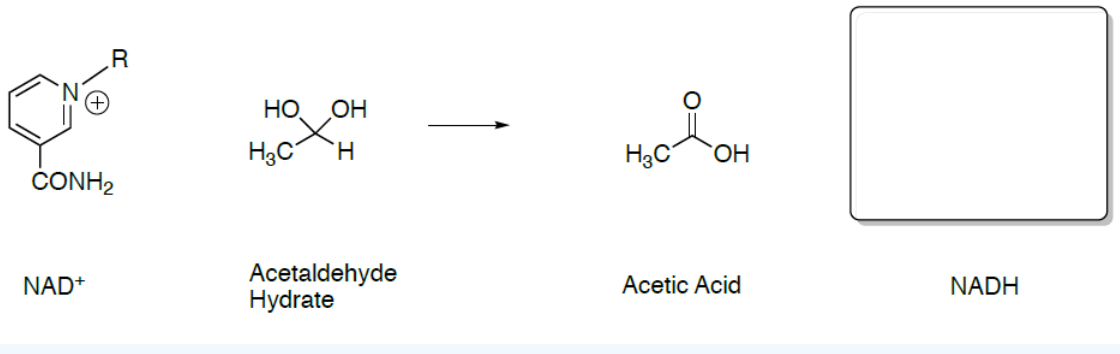
Propose a mechanism for the conversion of ethanol to acetaldehyde (reverse of the reduction done by yeast) utilizing NAD^+.



In the second step, acetaldehyde forms a hydrate which is then converted to acetic acid.

? Exercise 1.6.7

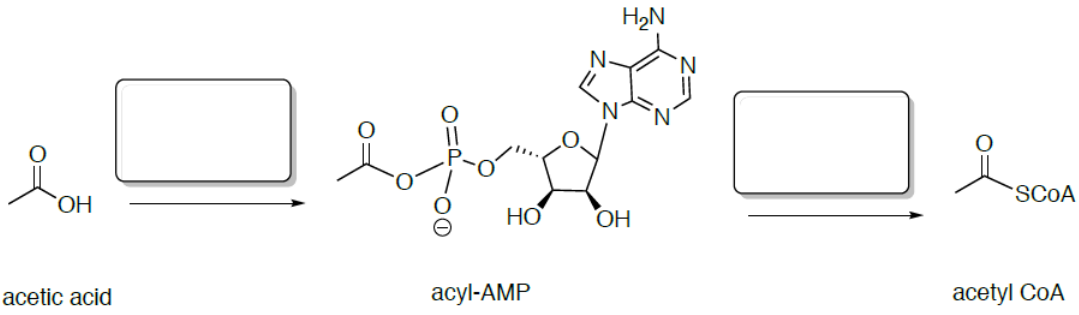
Propose a mechanism for the conversion of acetaldehyde to acetic acid utilizing NAD^+.



In the third step, acetic acid is converted to acetyl CoA for use in the TCA Cycle.

? Exercise 1.6.8

Propose the missing biological 'reagents' for this conversion.



Sources

1. Christopher Anthony, Quinoprotein Catalyzed Reactions, *Biochem J.*, **1996**, *320*, 697-711
2. Gómez-Manzo, et. al., The Oxidative Fermentation of Ethanol, *Int J Mol Sci.* **2015**, *16*(1), 1293–1311.
3. Mamlouk and Gullo, Acetic Acid Bacteria: Physiology and Carbon Sources Oxidation, *Indian J. Microbiology*, **2013**, *53*(4) 377-384.
4. Mas, et. al., Acetic Acid Bacteria and the Production and Quality of Wine Vinegar, *Scientific World Journal*, **2014;** *2014*, 1-6