FDE 328
INDUSTRIAL MICROBIOLOGY

Acetic Acid Fermentation Pathway

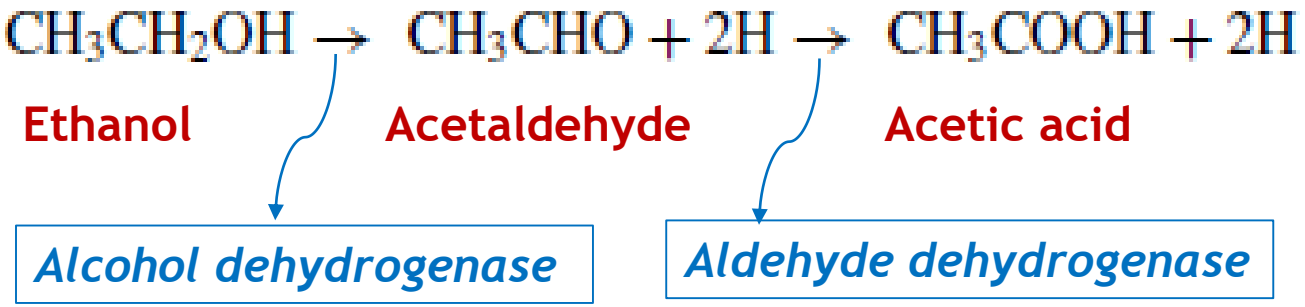
- Acetic acid fermentation is the biochemical process by which *Acetobacter* spp. oxidise ethanol into acetic acid **under strict conditions of aerobiosis**.
- Two genera of acetic acid bacteria are known in the production of vinegar: ***Acetobacter*** and ***Gluconobacter***.
- The acetic acid pathway used by *Acetobacter, Gluconobacter* and other acetic acid bacteria is an example of what biochemists refer to as **an incomplete oxidation**.
- In most oxidative pathways (e.g., the Krebs or citric acid cycle), organic substrates are ordinarily oxidized all the way to CO_2 and H_2O.
- However, in the vinegar fermentation, acetic acid bacteria usually oxidize the substrate, ethanol, only to acetic acid. There are exceptions that exist where complete oxidation to CO_2 can occur.

Acetic Acid Fermentation Pathway (Oxidation of Ethanol to Acetic Acid)

- Transformation of ethanol into acetic acid is the most well-known characteristic of acetic acid bacteria.

- This transformation involves two biochemical reactions: ethanol is first transformed into acetaldehyde in a reaction catalyzed by alcohol dehydrogenase and the acetaldehyde is then transformed into acetic acid by aldehyde dehydrogenase.

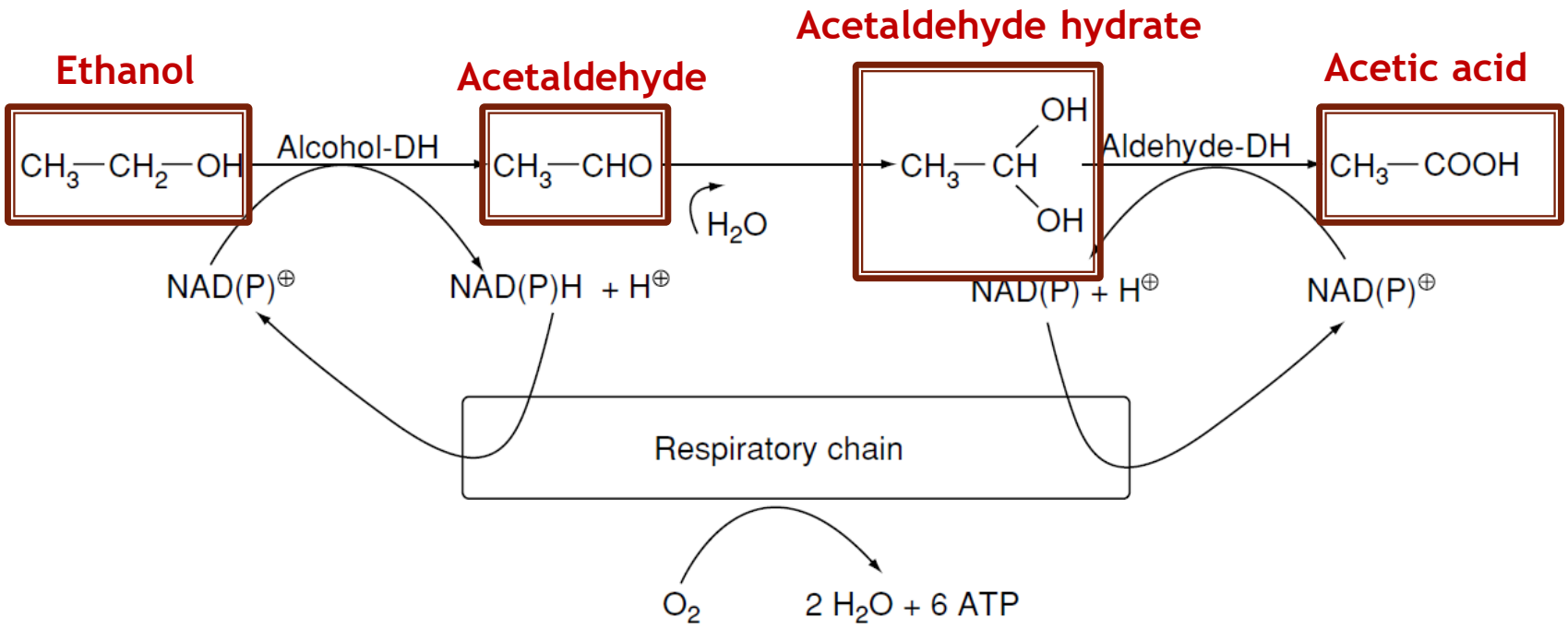
- Ethanol oxidation is performed by two sequential reactions:



Acetic Acid Fermentation Pathway (Oxidation of Ethanol to Acetic Acid)

- Briefly, the acetic acid pathway consists of just two main steps.
- First, ethanol is oxidized to acetaldehyde.
- Then, the acetaldehyde is oxidized to acetic acid.
- An intermediate step, in which acetaldehyde hydrate is formed, may also occur.
- Oxygen is required as the terminal electron acceptor.
- It should also be noted that the conversion of ethanol to acetic acid occurs on an equimolar basis, such that after the reactions are complete, the final concentration of acetic acid will be equal to that of the ethanol in the starting material (assuming negligible loss from evaporation).
- Only a minor amount of the carbon from ethanol is used for biomass or converted to other products.

Acetic Acid Fermentation Pathway (Oxidation of Ethanol to Acetic Acid)



Acetic Acid Fermentation Pathway

- The biochemical and physiological processes involved in the acetic acid fermentation are quite unlike those described for the lactic and ethanolic fermentations.
- The lactic and ethanolic fermentations are anaerobic fermentations, whereas the acetic acid fermentation is performed by obligate aerobes.
- The acetic acid pathway yields energy, but not by substrate level phosphorylation.
- Instead, the oxidation reactions are coupled to the respiratory chain that generates ATP via electron transport and oxidative phosphorylation reactions.
- Finally, the acetic acid fermentation is unique in that both the substrate (ethanol) and product (acetic acid) are toxic.

Acetic Acid Fermentation Pathway

*The two main enzymes involved in the acetic acid fermentation are alcohol dehydrogenase (AlDH) and acetaldehyde dehydrogenase (AcDH).

*These enzymes are the primary dehydrogenases responsible for nearly all of the detectable oxidation activity.

- Finally, as I noted earlier, the acetic acid pathway is usually considered an incomplete oxidation.
- This is because the substrate, ethanol, is only partially oxidized and the acetic acid that is formed is not oxidized further.
- However, while this is true for some acetic acid bacteria, such as *Gluconobacter* (a member of the so-called suboxydans group), most species of *Acetobacter* can oxidize acetic acid, provided conditions are suitable.
- The latter include bacteria of the oxydans group, represented by the common vinegar-producing species *A. aceti* and *A. pasteurianus*.
- The absence of ethanol in the medium is the main condition necessary for acetic acid oxidation.
- Ethanol apparently represses synthesis of citric acid cycle enzymes.
- When ethanol is absent, those enzymes are induced and complete oxidation of acetic acid to CO_2 and H_2O can occur.
- Of course, in actual vinegar production, this so-called **over-oxidation** of acetic acid is undesirable, because it causes the literal disappearance of the end product to the atmosphere.
- Thus, this is likely one reason why vinegar fermentations have historically been conducted in a semi-continuous mode, in which a minimum amount of ethanol is always present.

Overoxidation

- In addition to their ability to oxidise ethanol, *Acetobacter* and *Gluconacetobacter* species can further oxidise acetic acid to CO_2 and H_2O, generating the so-called **acetate overoxidation**, that is carried out by the tricarboxylic acid cycle when there is a high level of dissolved oxygen and no ethanol in the medium.

- Strains of *Gluconobacter* are not able to overoxidise because of nonfunctional α-ketoglutarate dehydrogenase and succinate dehydrogenase of tricarboxylic acid cycle; they can only oxidise ethanol to acetic acid.

- Because entry into the acetate cycle is inhibited by the presence of ethanol, it is essential to maintain a low concentration of ethanol in the presence of acetic acid bacteria to prevent this full oxidation.

- **In order to prevent over-oxidation, ethanol concentrations between 0.5 and 1% are regularly maintained in vinegars**.

Acetic Acid Bacteria (AAB)

- The acetic acid bacteria are mesophilic obligate aerobes that oxidize sugars, sugar alcohols, and ethanol, with the production of acetic acid as the major end product.

- During acetic acid production, ethanol is almost quantitatively oxidized to acetic acid.

- AAB exhibit resistance to high acetic acid concentrations and low pH.

- Although acetic acid bacteria are feared among oenologists because of their negative effects on grapes and on wine in general, they are the main agents in the production of vinegar.

Acetic Acid Bacteria (AAB)

Strains of acetic acid bacteria to be used in industrial production should

a) tolerate high concentrations of acetic acid;
b) require small amounts of nutrient;
c) not overoxidize the acetic acid formed;
d) be high yielding in terms of the acetic acid produced.

- **Over-oxidation** can occur and it is undesirable. In over-oxidation acetic acid is converted to CO_2 and H_2O. It occurs when there is a lack or low level of alcohol.