CHAPTER III: CARBOHYDRATE CATABOLISM

2.4. Krebs Cycle (Tricarboxylic Acid Cycle "TCA" or Citric Acid Cycle)

In the presence of oxygen, strict or facultative aerobic microorganisms ensure the complete oxidation of glucose. The pyruvate formed is further oxidized through the Krebs cycle and the glyoxylate shunt.

The Krebs cycle represents the aerobic oxidation pathway of acetate originating not only from glycolysis or from the hexose monophosphate pathway, but also from the β-oxidation of fatty acids. Its enzymatic components participate directly or indirectly in the degradation of the carbon skeleton of most amino acids. In addition, the cycle provides precursor compounds required for several biosynthetic reactions.

There are noticeable differences among organisms. In the "classical" cycle, malate is oxidized to oxaloacetate by an NAD-dependent malate dehydrogenase, as observed in *Escherichia coli*. In organisms such as *Serratia* or *Pseudomonas*, however, a dehydrogenase directly linked to cytochromes is present (Fig. 25).

Each turn of the cycle produces, from acetate, two molecules of CO_2 and eight reducing equivalents (H^+, e^-), in the form of **2 $NADH_2$, 1 $NADPH_2$, and 1 $FADH_2$**. These electrons and protons are transferred to oxygen through the respiratory chain. A maximum of **three ATP molecules** can be generated per pair of electrons transferred between NADH and oxygen.

Consequently, the overall yield per mole of glucose oxidized through glycolysis and the Krebs cycle can reach a theoretical maximum of **38 ATP molecules**.

The Krebs cycle cannot operate under anaerobic conditions because **succinate dehydrogenase** and **α-ketoglutarate dehydrogenase** become inactive. Nevertheless, certain reactions may still occur from oxaloacetate to succinate (a reductive branch operating in the reverse direction with the involvement of fumarate reductase) and toward α-ketoglutarate (an oxidative branch), as observed in *Escherichia coli*.

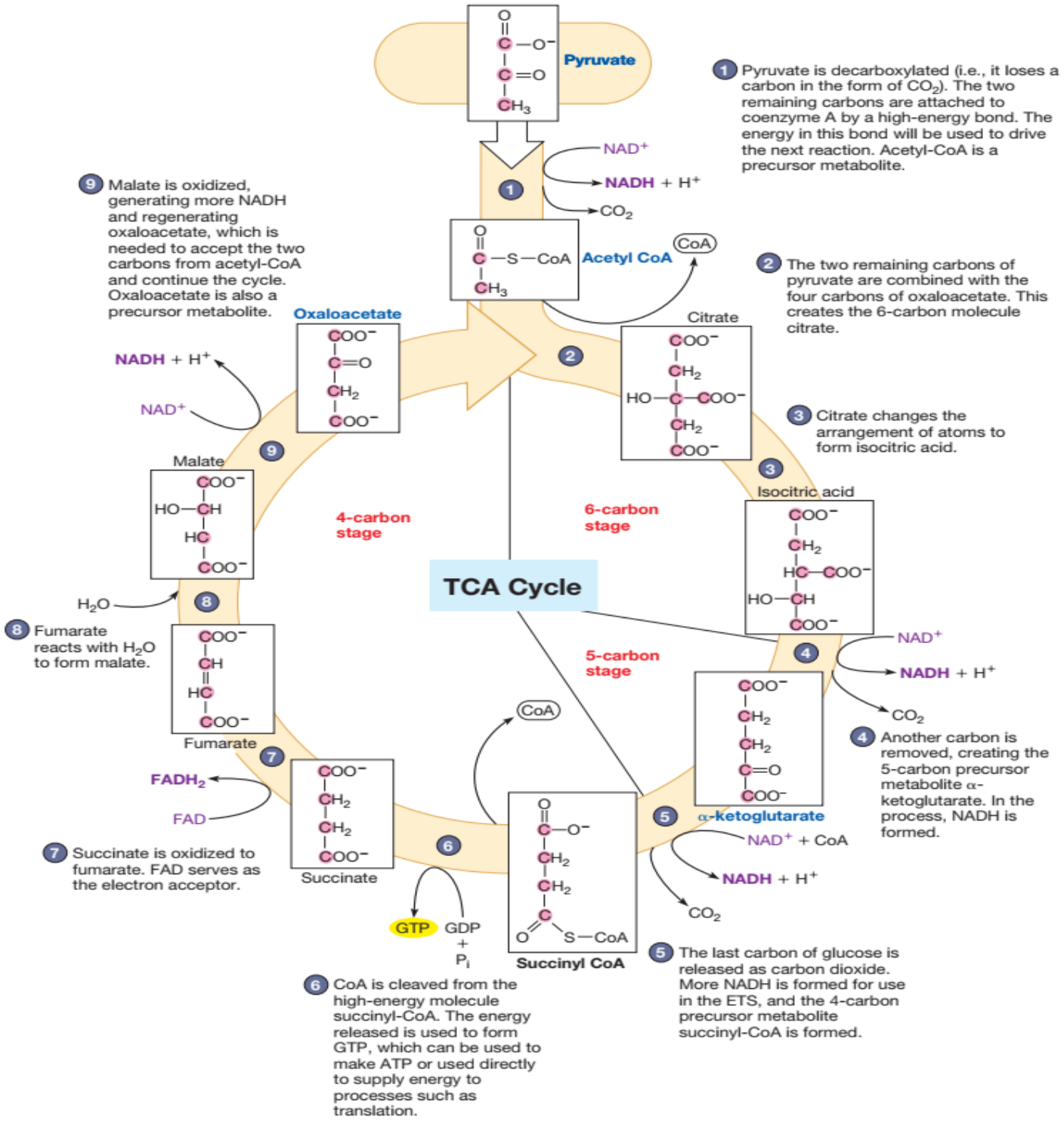


Figure 25: Tricarboxylic Acid Cycle.

[The TCA cycle is linked to glycolysis by a connecting reaction catalyzed by the pyruvate dehydrogenase complex. The reaction decarboxylates pyruvate (removes a carboxyl group as CO2) and generates acetyl-CoA. The cycle may be divided into three stages based on the size of its intermediates. The three stages are separated from one another by two decarboxylation reactions. Precursor metabolites, carbon skeletons used in biosynthesis, are shown in blue. NADH and FADH2 are shown in purple; all can transfer electrons to the electron transport chain (ETC)].

The first step of this process involves a multienzyme system known as the **pyruvate dehydrogenase complex**, which consists of three enzymes acting sequentially to catalyze the **oxidative decarboxylation of pyruvate into acetyl-CoA**. This complex oxidizes and cleaves pyruvate to produce carbon dioxide and acetyl-coenzyme A.

$$\text{Pyruvate} + NAD^+ + CoA \rightarrow \text{Acetyl-CoA} + NADH + H^+ + CO_2$$

Acetyl-CoA is a high-energy molecule because a **sulfhydryl (–SH) group** forms a high-energy thioester bond linking acetic acid to coenzyme A. Acetyl-CoA then enters the **tricarboxylic acid (TCA) cycle**, also known as the **Citric acid cycle** or **Krebs cycle**.

In the first reaction, **acetyl-CoA**, under the action of the enzyme ***citrate synthase***, condenses with a four-carbon intermediate, **oxaloacetate**, forming **citrate**, a six-carbon molecule.

$$\text{Acetyl-CoA} + H_2O + \text{Oxaloacetate} \rightarrow \text{Citrate} + CoA$$

Citrate (a tertiary alcohol) is then rearranged by the enzyme ***aconitase*** to produce **isocitrate**, a secondary alcohol that is more easily oxidized.

Isocitrate is subsequently oxidized and decarboxylated by ***isocitrate dehydrogenase***, producing **α-ketoglutarate (five carbons)**. This overall reaction occurs in two steps:

$$\text{Isocitrate} + NADP^+ / NAD^+ \rightleftarrows \text{Oxalosuccinate} + NADPH / NADH + H^+$$

$$\text{Oxalosuccinate} \rightleftarrows \alpha\text{-Ketoglutarate} + CO_2$$

Next, **α-ketoglutarate** is converted by the ***α-ketoglutarate dehydrogenase*** **complex** into **succinyl-CoA** (four carbons)**,** a molecule containing a high-energy bond. At this stage, <u>two NADH molecules have been produced and two carbons have left the cycle as CO_2**.**</u>

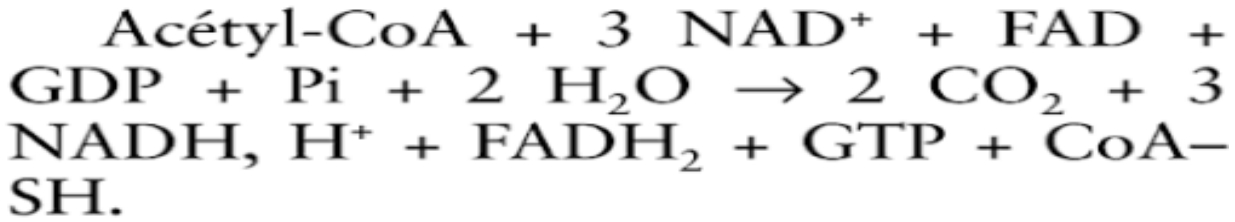
The cycle continues with the conversion of **succinyl-CoA** into **succinate** by the enzyme ***succinyl-CoA ligase***. The high-energy bond of succinyl-CoA is broken, and the released energy is used to generate <u>GTP through substrate-level phosphorylation</u>. GTP is also a high-energy molecule and is functionally equivalent to ATP.

Next, **succinate** is dehydrogenated into **fumarate** by the enzyme ***succinate dehydrogenase***, which uses FAD as a cofactor. This reaction generates $\mathbf{FADH_2}$.

In the penultimate reaction, **fumarate** is hydrated to **L-malate** by the enzyme ***fumarase***. In the final step, **malate** is oxidized to regenerate **oxaloacetate**. As long as acetyl-CoA continues to enter the cycle, the reactions proceed repeatedly.

Overall, the **TCA cycle** produces **two CO_2 molecules, three NADH, one $FADH_2$,** and **one GTP** per **molecule of acetyl-CoA** oxidized.

The enzymes of the TCA cycle are widely distributed. In **prokaryotes**, they are located in the **cytoplasm**, while in **eukaryotes** they are found in the **mitochondria**. A complete TCA cycle is functional in many aerobic bacteria, free-living protists, and fungi. This is not surprising, since the cycle is a major source of energy. Even microorganisms lacking a complete TCA cycle usually possess most of its enzymes because the cycle also provides **carbon skeletons required for biosynthesis**.



2.5. Glyoxylate Shunt

Some microorganisms (such as *Escherichia coli*, many molds, and several *Pseudomonas* species) can grow using **acetate** as their sole carbon and energy source. These organisms possess all the enzymes of Krebs cycle but also contain two additional enzymes:

- **Isocitrate lyase**, which cleaves isocitrate into **succinate** and **glyoxylate**
- **Malate synthase**, which condenses **glyoxylate** with **acetyl-CoA** to form **malate**

The glyoxylate shunt does not produce biologically usable energy. It operates only when microorganisms grow on **acetate**, because glucose represses the synthesis of the enzymes involved**.**

During growth on acetate, cells **decarboxylate oxaloacetate** to produce **phosphoenolpyruvate**, which serves as the starting point for the **biosynthesis of hexoses and pentoses**.

Overall equation:

$$2\ \text{Acetyl-CoA} + FAD^+ + 2NAD^+ + 3H_2O \rightarrow \text{Oxaloacetate} + 2\text{CoA} + FADH_2 + 2NADH + 2H^+$$

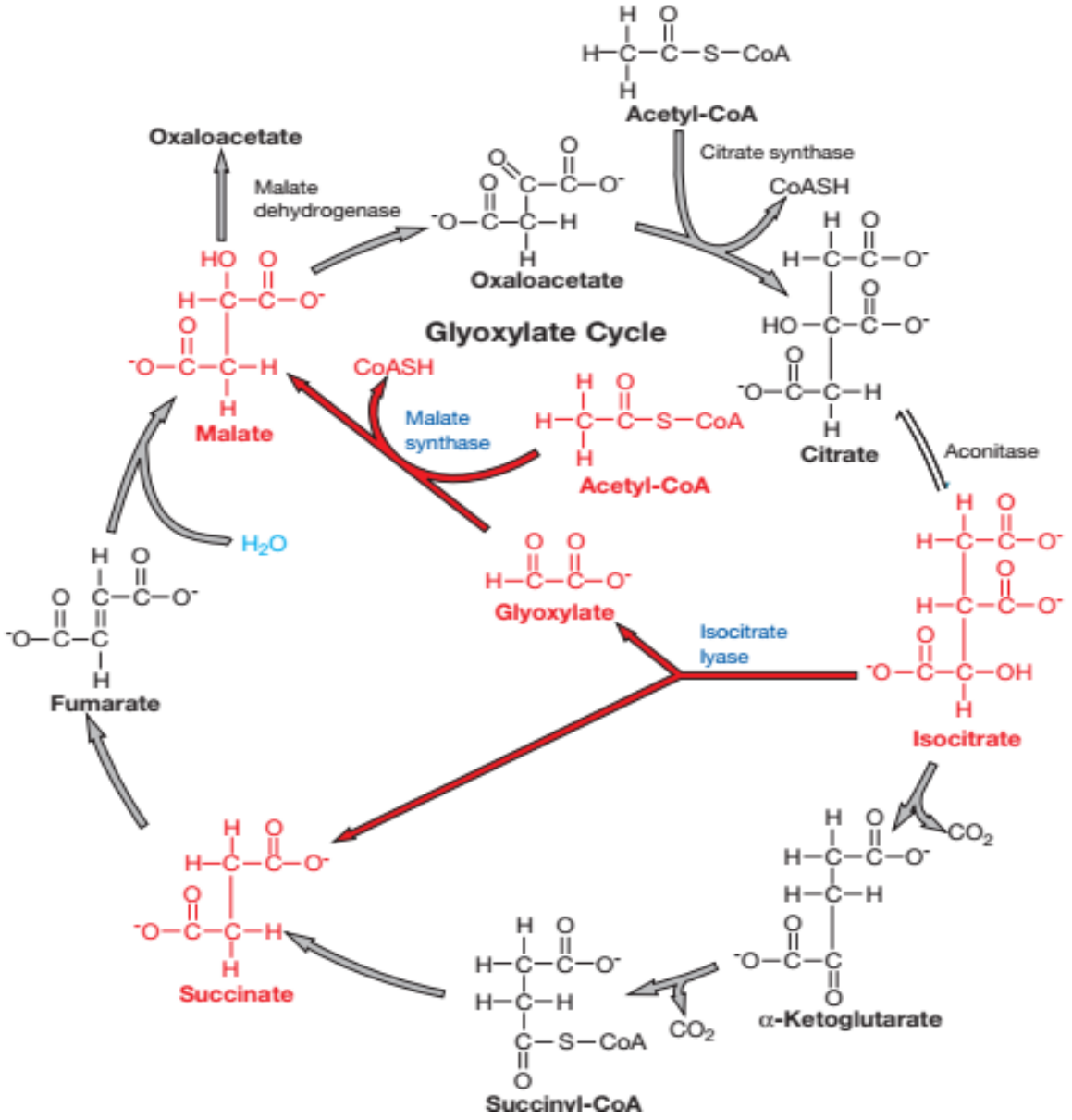


Figure 26: Glyoxylate Cycle.

[The reactions and enzymes unique to the cycle are shown in red. The tricarboxylic acid cycle enzymes that have been bypassed are at the bottom].

2.6. ATP yield during aerobic respiration

It is possible to estimate the number of ATP molecules synthesized per NADH or $FADH_2$ oxidized by the electron transport chain. During aerobic respiration, a pair of electrons from NADH is donated to the electron transport chain and ultimately used to reduce an atom of oxygen to H_2O. This releases enough energy to drive the synthesis of three ATP. This is referred to as the

phosphorus to oxygen (P/O) ratio because it measures the number of ATP (phosphorus) generated per oxygen (O) reduced (Fig. 27). Because $FADH_2$ has a more positive reduction potential than NADH, electrons arising from its oxidation flow down a shorter chain, releasing less energy. Thus, while the P/O ratio for NADH is 3, only two ATP can be made from the oxidation of a single $FADH_2$.

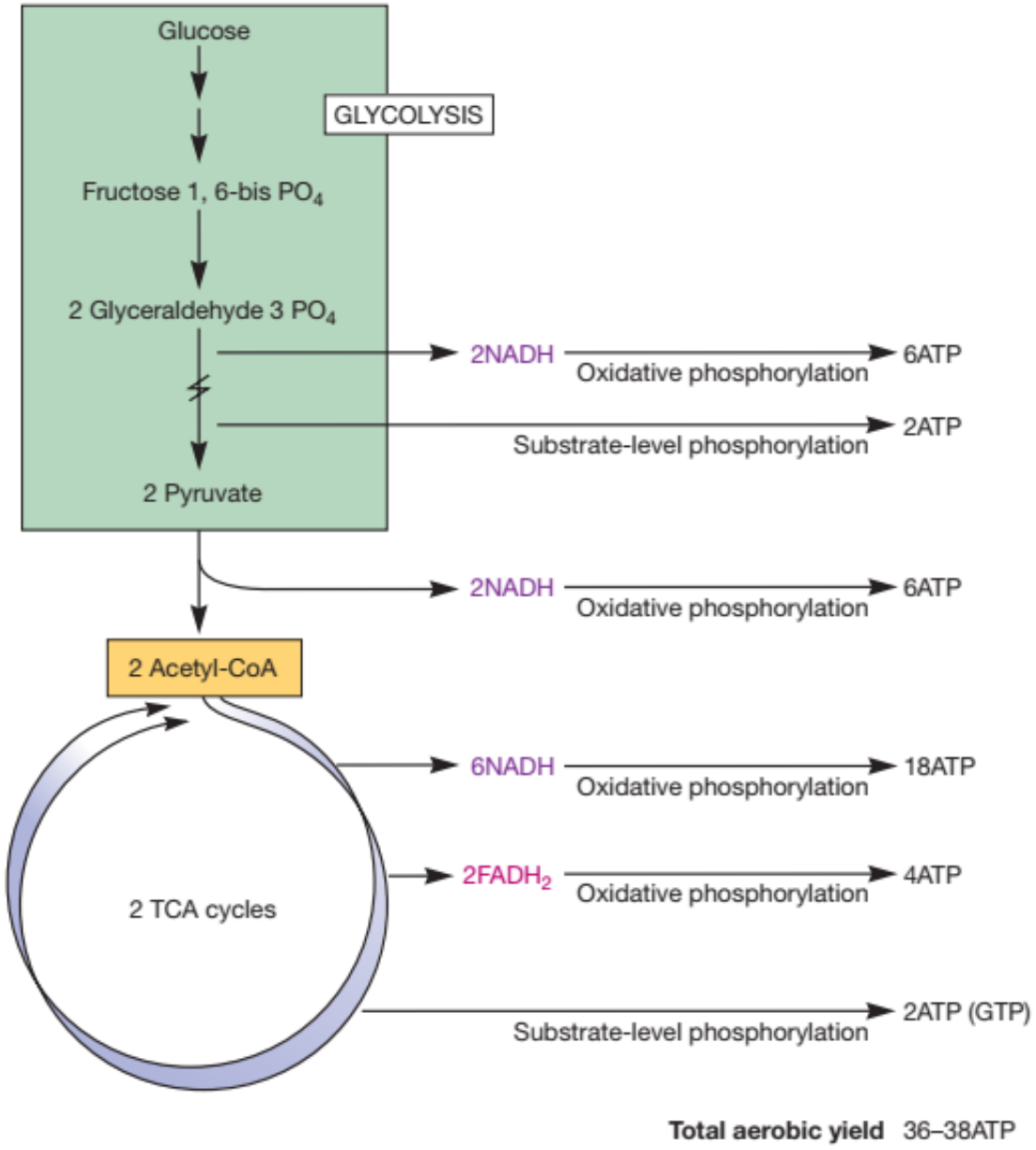


Figure 27: Maximum theoretic ATP yield from aerobic respiration.

[actual yield is probably significantly less and varies between eucaryotes and procaryotes and among procaryotic species].

2.7. Fermentations derived from Krebs cycle and Glyoxylate shunt

These fermentations are mainly aerobic processes carried out by molds. They result in the formation of various **organic acids**, which are metabolic products derived directly from the Krebs cycle, the glyoxylate shunt, or their transformation products.

These acids accumulate when the normal operation of the cycle is interrupted. Such interruption may occur due to changes in environmental conditions, including:

- Variation in pH ;
- Presence of enzyme inhibitors that block the normal transformation of the produced compound;
- Mutations affecting genes controlling these enzymes.

The organic acids produced through these fermentations are diverse and include: Citric acid, Itaconic acid, Fumaric acid, Oxalic acid, Malic acid, Glutaric acid, Succinic acid, Epoxysuccinic acid, …

Example:

a. Citric acid production

Citric acid is widely used in the food and pharmaceutical industries as an acidifying agent, emulsifier, antioxidant, and chelating agent. It is produced by strains of *Aspergillus niger* or *Aspergillus wentii* (Fig. 28).

Several industrial processes are used:

- *Koji fermentation*, traditionally carried out in Japan using moist wheat bran;
- *Surface fermentation* on molasses ;
- *Submerged (agitated) fermentation* on molasses.

Citric acid accumulation is achieved either by using **mutant strains** or by modifying the culture medium (for example, phosphate limitation or adjustment of mineral salts). **Iron inhibits production**, whereas **copper stimulates it**.

During fermentation, aconitase activity disappears, and isocitrate lyase and isocitrate dehydrogenase are inhibited. In most cases, the fermentation occurs in two stages: initially there is mycelial growth, followed by cessation of growth and accumulation of citric acid.

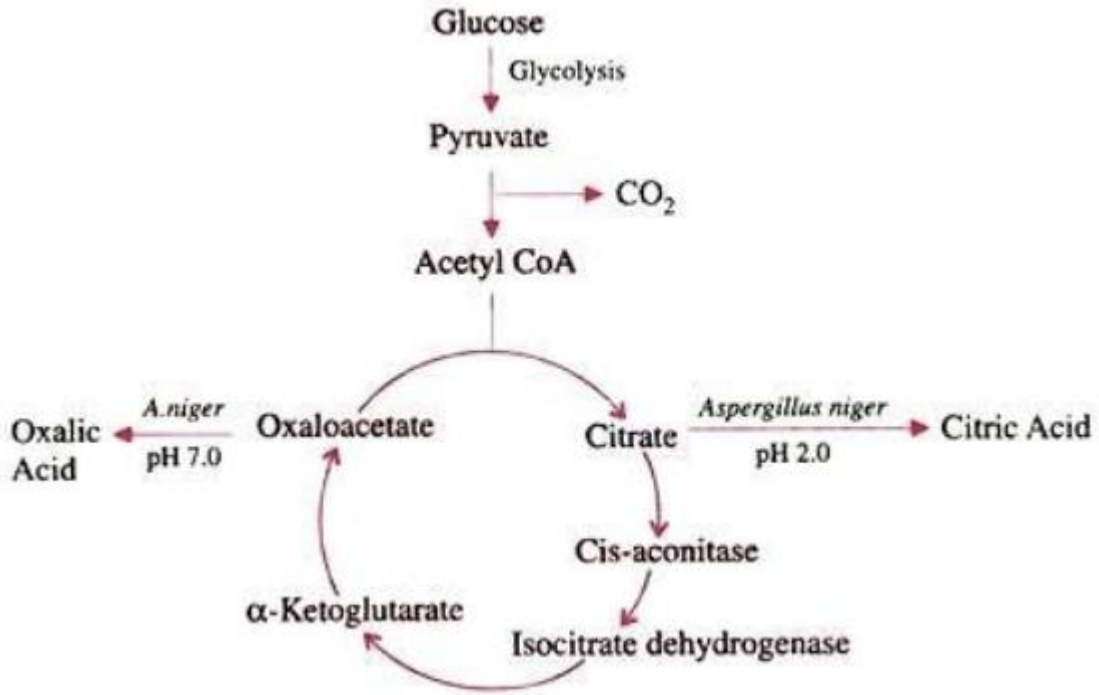


Figure 28: Biochemical pathway of oxalic acid and citric acid

b. Fumaric acid production

Fumaric acid, used in food industry and particularly in polyester resin production, is produced by various strains of *Aspergillus* and *Rhizopus*. From 120 g/L of glucose, *Rhizopus arrhizus* can produce about 97 g/L of fumaric acid.

Adequate **aeration** of the culture is essential for fumaric acid accumulation. Without aeration, *ethanol and CO_2* become the main fermentation products.

Fermentation of glucose in an **aerated and agitated medium containing $CaCO_3$** leads to crystallization in the form of **calcium fumarate**.